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SUPERCRITICAL FLUID EXTRACTION OF PARTICULATE AND ADSORBENT MATERIALS: PART II

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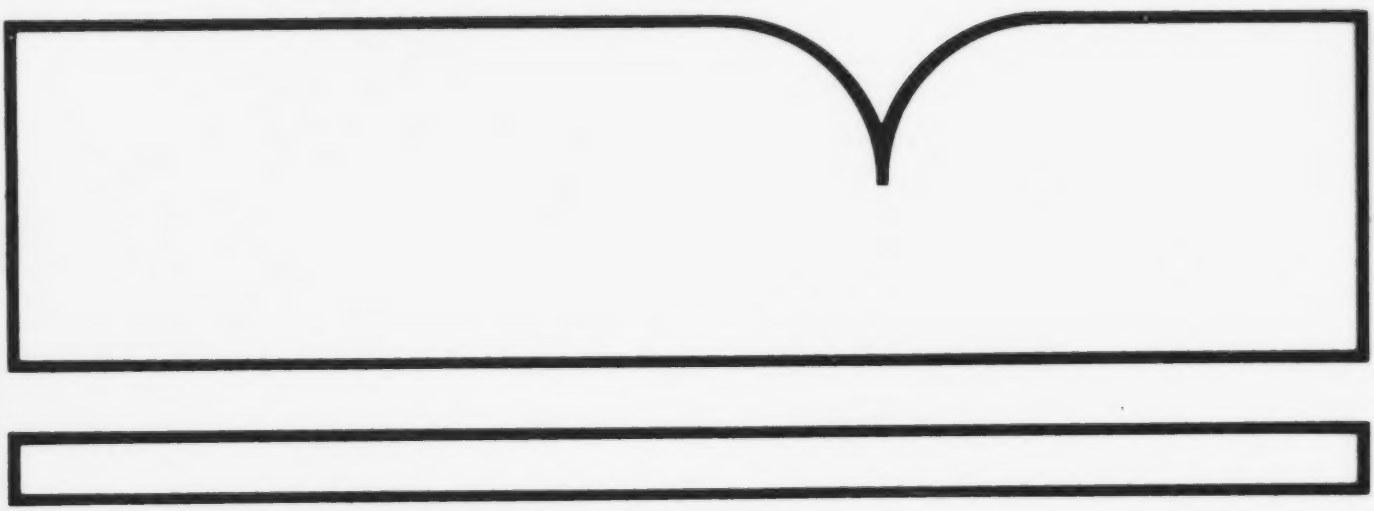

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SUPERCRITICAL FLUID EXTRACTION OF PARTICULATE AND ADSORBENT MATERIALS: PART II

by

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16. ABSTRACT

The physical properties of supercritical fluids allow similar solvent strengths as liquids, but with higher diffusion coefficients, lower viscosities and an extended temperature range which provides the potential for more rapid and efficient extraction rates than possible with liquids. This report describes expanded studies conducted to evaluate the applicability and efficiency of analytical supercritical fluid extraction and related methodologies. These studies included the development of quantitative off-line supercritical fluid extraction methodology and a comparison to traditional Soxhlet extraction, the development and evaluation of on-line supercritical fluid extraction-gas chromatography for combined sample preparation and analysis, and direct supercritical fluid extraction-mass spectrometry for the monitoring of specific extraction profiles as a function of time. The sample matrices included an air particulate sample and XAD-2 resin, polyurethane foam, and Spherocarb® adsorbents that were spiked with various model compounds. Carbon dioxide, isobutane, and methanol modified (20 mole %) carbon dioxide were utilized as supercritical fluid systems. Related studies on the evaluation of the quantitative analysis capability of a fluorescence detection supercritical fluid chromatography method and the development of viable solute focusing methods for capillary supercritical fluid chromatography were also conducted.

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FOREWORD

Measurement and monitoring research efforts are designed to anticipate environmental problems, to support regulatory actions by developing an in-depth understanding of the nature of processes that impact health and the ecology, to provide innovative means of monitoring compliance with regulations, and to evaluate the effectiveness of health and environmental protection efforts through the monitoring of long-term trends. The Environmental Monitoring Systems Laboratory, Research Triangle Park, North Carolina, has responsibility for: assessment of environmental monitoring technology and systems for air, implementation of agency-wide quality assurance programs for air pollution measurement systems, and supplying technical support to other groups in the Agency including the Office of Air and Radiation, the Office of Toxic Substances, and the Office of Solid Waste.

The determination of human exposure to toxic organic compounds is an area of increasing importance to EPA. Many of these compounds are extremely difficult or impossible to quantify in environmental samples with current analytical methodology. The use of supercritical fluids as extraction solvents and chromatographic mobile phases has significant potential for extending current methodology to these more challenging samples. This report summarizes recent work in the development of such supercritical fluid-based analytical methodology.

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ABSTRACT

The potential utility of supercritical fluid extraction for analytical sample preparation and the development of related methods of analysis were investigated. These studies included off-line supercritical fluid extraction of adsorbents and particles, on-line supercritical fluid extraction-gas chromatography, and direct supercritical fluid extraction-mass spectrometry. Related studies on the evaluation of the quantitative analysis capability of a fluorescence detection supercritical fluid chromatography method and the development of viable solute focusing methods for capillary supercritical fluid chromatography were also conducted.

Off-line supercritical fluid extractions using various fluid systems were evaluated and compared to Soxhlet extraction for the recovery of low concentrations (ppm) of model compounds from selected adsorbents. Higher molecular weight (228-450 daltons) polycyclic aromatic compounds were extracted from XAD-2 resin, polyurethane foam (PUF), and Spherocarb® adsorbents using carbon dioxide, isobutane, and methanol-modified (18.2 mole percent) carbon dioxide fluid systems. Air particulate matter was also used as a complex matrix for evaluation of the supercritical fluid extraction methods. Instrumentation was designed to allow gram quantities of the matrix to be extracted at pressures up to 400 bar and temperatures to 235°C with collection of the effluent in sealed flasks. Supercritical fluid extraction (SFE) provided very rapid (~ 30 minutes) extraction with comparable efficiency to the Soxhlet methods, and both more rapid and efficient extractions appear feasible. The more polar carbon dioxide-methanol fluid system gave higher extraction efficiencies for the more polar adsorbates and the isobutane system was more efficient for the higher molecular weight and less polar compounds.

Analytical methodology combining on-line supercritical fluid extraction with high resolution capillary gas chromatography for automated sample preparation and analysis is described. The supercritical fluid extracts were decompressed through a restrictor to deposit and concentrate the analytes at the inlet of a standard capillary gas chromatography column for subsequent analysis. This methodology allows several modes of operation including quantitative extraction of all analytes from a sample matrix, quantitative extraction and concentration of trace analytes, selective extractions at various solvating powers to obtain specific fractions, or multiple-step extractions at various pressures for qualitative characterizations. These modes of operation are described and the potential usefulness of this methodology for sample extraction and selective fractionation demonstrated using a standard polycyclic aromatic hydrocarbon mixture and two complex sample matrices.

Direct supercritical fluid extraction-mass spectrometry was utilized to monitor the individual extraction profiles of each model compound as they were extracted from XAD-2 resin, PUF, and Spherocarb® adsorbents with carbon dioxide, isobutane, and methanol-modified (18.2 mole %) carbon dioxide fluid systems. Somewhat different extraction behaviors (threshold pressures, extraction rate, solubility, decay curves, etc.) were observed among the model compounds and from the various adsorbents. Extraction profiles for Spherocarb® could not be constructed since only very low signal levels were detected.

The quantitative analysis capability of the fluorescence detection capillary supercritical fluid chromatography system that was developed to obtain high sensitivity detection when using fluids

that are not compatible with flame ionization detection was evaluated. The injection and detection reproducibility was sufficient to allow calibration plots to be generated with less than an 8 % standard error. Linear response was obtained over the narrow range investigated. The model compound concentration levels determined from various extracts agreed well with values determined by capillary gas chromatography. With the exception of obvious problem injections, less than 15 % relative differences were obtained among replicate analyses. Poor reproducibility was ascribed to injection problems.

Preliminary studies were conducted to develop viable solute focusing methods for capillary supercritical fluid chromatography similar to cryogenic trapping in gas chromatography. One approach that proved moderately successful was to heat a short length of the capillary column inlet to a higher temperature during injection. This significantly reduced the fluid density in a short length of the column which aided focusing of the solutes in a narrow injection band.

This report was submitted in fullfillment of Interagency Agreement DW899 30650-01 by Pacific Northwest Laboratory under the sponsorship of the U.S. Environmental Protection Agency through a Related Services Contract with the U. S. Department of Energy under Contract DE-ACO6-76RLO-1830. This report covers a period from February 1, 1985 to January 31, 1986, and work was completed as of January 31, 1986.

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SECTION 1

INTRODUCTION

Numerous methods and combinations of methods with varying degrees of complexity are currently employed to prepare samples for analysis. Less complex and more rapid preparation procedures resulting in improved sensitivities would be desirable in many cases, particularly for small samples or low concentrations of analytes. Supercritical fluid extraction (SFE) has been successfully used for a large number of large-scale chemical processing applications and is currently attracting renewed interest (1-3). However, only limited attention has been given to supercritical fluid extraction methods for analytical applications (4,5). A number of potential advantages including more rapid extraction rates, more efficient extractions, increased selectivity, potential for combined analyte fractionation in conjunction with extraction, and the capability for direct continuous on-line analysis, such as with mass spectrometry (6,7) or periodic gas chromatographic analysis are possible with SFE compared to conventional liquid extraction methods. The goals of this study were to determine the potential utility of supercritical fluid extraction for analytical sample preparation and to develop improved methods of analysis utilizing these and other supercritical fluid analytical methodologies.

The potential advantages of SFE accrue from the properties of a solvent at temperatures and pressures above its critical point. At elevated pressure this single phase will have properties which are intermediate between those of the gas and liquid phases and are dependent on the fluid composition, pressure, and temperature. The compressibility of supercritical fluids is large just above the critical temperature and small changes in pressure result in large changes in density of the fluid (8). The density of a supercritical fluid is typically 10^2 to 10^3 times greater than of the gas. Consequently, molecular interactions increase due to shorter intermolecular distances. However, the diffusion coefficients and viscosity of the fluid, although density dependent, remain more similar to that of a gas (8). The "liquid-like" behavior of a supercritical fluid results in greatly enhanced solubilizing capabilities compared to the subcritical gas, and higher diffusion coefficients, lower viscosity, and an extended temperature range compared to the corresponding liquid. These properties allow similar solvent strengths as liquids but with greatly improved mass transfer characteristics which provide the potential for both more rapid extraction rates and more efficient extraction due to better penetration of the matrix.

This report describes expanded studies conducted to evaluate the applicability and efficiency of analytical supercritical fluid extraction and related methodologies. The initial investigations involved in this project have been previously reported (9). The current studies included off-line supercritical fluid extraction (SFE) of adsorbents and particles, on-line supercritical fluid extraction-capillary gas chromatography (SFE-GC), and direct supercritical fluid extraction-mass spectrometry (SFE-MS). The quantitative analysis capability of the fluorescence detection supercritical fluid chromatography (SFC) system that was previously developed in this project was also evaluated. Preliminary studies aimed at developing viable solute focusing methods for capillary supercritical fluid chromatography similar to cryogenic trapping in gas chromatography were also conducted.

In the off-line SFE studies, various fluid systems were evaluated and compared to Soxhlet extraction for the recovery of low concentrations (ppm) of model compounds from selected adsorbents. Higher molecular weight (228-450 daltons) polycyclic aromatic compounds were extracted from XAD-2 resin, polyurethane foam, and Spherocarb adsorbents. Air particulate matter was also used as a complex matrix for evaluation of the supercritical fluid extraction methods. In addition to carbon dioxide, isobutane and methanol-modified (18.2 mole percent) carbon dioxide were used for SFE solvents. The instrumental considerations involved in the design of an analytical-scale SFE system were also addressed.

A logical extension of supercritical fluid extraction is to combine the process with a chromatographic analysis method. The variable solvating power of a supercritical fluid furnishes the mechanism for the selective extraction of the components of interest from the sample matrix and provides the basis for an automated method where sample preparation and analysis can be instrumentally linked. The instrumentation and methodology developed for the automated SFE-GC system and its application to sample fractionation and compound isolation are demonstrated using supercritical carbon dioxide extraction of two complex mixture samples and a standard mixture of polycyclic aromatic hydrocarbons (PAH).

SFE-MS was used to selectively monitor the total extraction effluent of spiked XAD-2 resin and PUF adsorbents using carbon dioxide, isobutane, and methanol-modified (18.2 mole %) carbon dioxide fluid systems. This allowed individual extraction profiles of each of the spiked compounds as a function of time to be obtained.

In addition to the above, other studies were conducted to evaluate the reliability of quantitative fluorescence SFC analyses and to develop the basis for necessary interface methods of combining supercritical fluid extraction with capillary supercritical fluid chromatography. This report describes several supercritical fluid-based analytical methodologies with significant potential for improved sample preparation and analysis.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions can be drawn as a result of the off-line supercritical fluid extraction studies conducted during this project:

- Optimized off-line analytical supercritical fluid extraction instrumentation and methods were developed which allowed high molecular weight polycyclic aromatic compounds to be successfully extracted and recovered from adsorbent matrices. Utilization of a reciprocating pump provided the potential for unlimited volumes of fluid to be used in an extraction, and collection of the extraction effluent in a sealed liquid- nitrogen-cooled flask prevented analyte losses during fluid decompression due to aerosol formation.

- Severe analyte losses can result from aerosol formation during decompression of the extraction fluid. When an open collection method was used under conditions where aerosol formation was expected to be inhibited (in the case of low temperature expansion where a two-phase solvent product was expected or when a polar liquid-modified fluid was used), significantly higher extraction recoveries were obtained.

- High recoveries of all the model compounds from the XAD-2 and PUF adsorbents were achieved by Soxhlet extraction.

- In general, the SFE methods provided extraction efficiencies comparable to traditional Soxhlet methods, but with over an order of magnitude increase in extraction speed. Supercritical fluid extractions were accomplished in 30-45 minutes compared to 16 hours for Soxhlet extraction, and faster extractions appear feasible. The more polar and higher molecular weight analytes were more difficult to extract from the adsorbents with a given volume of fluid. The modified carbon dioxide fluid and higher critical temperature isobutane systems provided higher extraction efficiencies than carbon dioxide.

- The model compounds were more easily extracted from the PUF matrix than the XAD-2 resin. Higher levels of background components were also extracted from the PUF matrix and the matrix itself appeared to be slightly soluble in methanol-modified carbon dioxide. Other than this case, the various adsorbents did not appear to be affected by supercritical fluid extraction.

- Supercritical fluid extraction of the highly adsorptive Spherocarb® matrix with isobutane and the methanol-modified carbon dioxide fluids provided more efficient extraction recoveries than achieved by Soxhlet extraction. However, only low levels of the less polar analytes were recovered.

- Supercritical fluid extraction also provided more rapid and improved extraction efficiency of PAH material from the Urban Dust matrix compared to Soxhlet extraction. The concentration levels of selected compounds obtained from these analyses exhibited reasonable agreement with NBS certified values.

The following conclusions can be drawn as a result of the on-line supercritical fluid extraction studies conducted during this project:

- On-line supercritical fluid extraction-gas chromatography provides the potential for combined sample preparation and analysis. In addition to completely automated operation, rapid analyses and high sensitivities can be achieved with this methodology.

- The selectivity obtainable with the wide range of solvent powers available with supercritical fluid extraction provides the potential for fractionation of complex samples and isolation of specific analytes from a matrix. The viability of the on-line SFE-GC concept, its qualitative operation for sample extraction from adsorbent matrices, and its potential for fractionation and isolation of specific components from more complex matrices was demonstrated.

- The quantitative extraction and transfer of PAH analytes from an XAD-2 resin to the gas chromatographic column for analysis was demonstrated. Quantitative extraction and transfer depends on solute solubility and requires an adequate volume of the supercritical fluid to be utilized.

The following conclusions can be drawn from the remainder of the studies conducted during this project:

- Direct supercritical fluid extraction-mass spectrometry provided a means of simultaneously monitoring the extraction profiles of individual model compounds from adsorbents, and was a useful tool for methods development with these techniques.

- The extraction behaviors of the model compounds from the various adsorbents with the different fluid systems were consistent with the off-line supercritical fluid extraction studies. The compounds were more soluble and were extracted more thoroughly and rapidly with supercritical isobutane than with carbon dioxide.

- The methanol-modified carbon dioxide fluid was utilized at subcritical temperature conditions, which resulted in a two-phase liquid-gas mixture rather than a supercritical fluid. Consequently, polar compounds capable of hydrogen bonding with liquid methanol were extracted more rapidly and more efficiently than the neutral compounds.

- The model compounds were extracted more easily from PUF than from XAD-2 with all the fluid systems utilized. The extraction profile intensities from PUF corresponded to the expected solubility orders (e.g., decreasing with decreasing vapor pressure) of the compounds. The model compounds were so strongly adsorbed to the Spherocarb® matrix that SFE-MS extraction profiles could not be obtained.

- The fluorescence detection supercritical fluid chromatography system provided adequate quantitative determinations with a combined uncertainty of approximately 15 %. Linear response of the neutral PAH model compounds was achieved over the narrow concentration range studied and sensitivies in the low picogram range could be obtained. Calibration plots with less than an 8 % standard error were constructed from replicate analyses at four concentration levels. Slightly better calibration was obtained from peak heights rather than peak area. Replicate analyses of standard mixtures and authentic sample extracts were reproducible to within approximately 15 %. Quantitative determinations of authentic sample extracts from parallel analyses utilizing capillary gas chromatography and the fluorescence detection SFC were generally consistent within a 20 % range. A significant portion of the quantitative variance can be attributed to injection problems.

- The basis for a successful solute focusing technique for capillary SFC was developed. The technique utilized a localized zone of lowered density created by heating a short length of the column inlet. Preliminary evaluations of other approaches utilizing a retention gap concept and a pressure gradient were also conducted.

Recommendations for related research and expanding the off-line supercritical fluid extraction studies are listed below:

- Since the overall supercritical fluid extraction rates appear to be limited by the solubility of the analytes in the extraction fluid rather than by desorption or mass transfer rates, more rapid and more efficient extractions should be feasible by developing methods to use larger fluid volumes at higher rates. Larger fluid volumes might be best obtained by recompression and recycling of the fluid and would also provide a basis for efficient sample fractionation.

- Additional studies need to be conducted to develop collection methods and apparatus that are more amenable to routine application.

- A more rigorous study of the chemical composition of the supercritical fluid extracts of NBS Urban Dust should be conducted. Detailed fractionations and analyses of the extracts would not only provide additional chemical composition information, but would provide additional insights on the extraction process.

Recommendations for related research and expanding the on-line SFE-GC and SFE-MS extraction studies are listed below:

- More rigorous quantitative studies using multiple analyses and a multi-level calibration with a variety of model compounds should be conducted to verify the performance of the on-line SFE-GC methodology.

- Methods of increasing the extraction fluid flow rate to allow larger sample matrices to be extracted rapidly should be investigated for the SFE-GC approach.

- The methanol-modified carbon dioxide SFE-MS studies should be expanded to examine extraction temperature effects and a comparison made of results obtained above the critical point to those obtained in this study using a subcritical two-phase mixture.

- Appropriate response factors should be obtained and quantitative SFE-MS extraction profiles obtained. The SFE-MS approach should be expanded to include a chromatographic separation step to allow concentration of trace components in the extraction effluent and provide separation of the components prior to MS analysis.

Further recommendations for research related to the other studies conducted during this project are listed below:

- The linearity of the fluorescence detector response should be evaluated over a much wider range, e.g., > 10^3, to establish analytical utility.

- Improved splitless injection methods should be developed to improve the accuracy and reproducibility of quantitative SFC analyses. Such improvements could include high-speed valve switching to effect lower volume injections and use of long retention gaps (>10 m) to aid solute focusing on the analytical column. The utilization of valves with 1/32" zero-dead-volume outlet fittings should also be investigated.

- Additional studies for the development of on-column solute focusing methods for capillary SFC should be continued. Such studies should include use of long retention gaps coupled with thermal focusing and the development of on-column solute concentration methods using extraction-injection and pressure focusing techniques.

SECTION 3

MATERIALS AND METHODS

ADSORBENTS AND PARTICLES

The following materials were used for adsorbents in the extraction studies: XAD-2 purified resin, 20/60 mesh (Alltech, Deerfield, IL), solvent cleaned polyurethane foam plugs (PUF) (General Metal Works, Cleaves, OH), and Spherocarb®, 80/100 mesh (Analabs, North Haven, CT). A National Bureau of Standards air particulate sample, Urban Dust SRM 1649, was also used for extraction.

Adsorbent Pretreatments

Purified XAD-2 resin was purchased that had been cleaned and evaluated by an EPA procedure established for the preparation of resins free of extractable organics (EPA-600/7-78-201). The polyurethane foam plugs (PUF) were cleaned using a procedure developed for the Methods Development Branch of the EPA. This procedure involved squeezing the PUF cartridges in two solvents. The PUF cartridges were first placed in toluene and squeezed ten times by using the base of a graduated cylinder. The process was repeated using acetone followed by drying in a nitrogen stream. The Spherocarb® was cleaned by thermal conditioning with a nitrogen flow for sixteen hours at 200°C.

The adsorbents were equilibrated in a low relativehumidity atmosphere (<10%) by storing the cleaned materials at room temperature in a closed desiccator containing freshly dried anhydrous calcium chloride and sodium sulfate. The adsorbents were placed in shallow dishes to allow maximum exposure and diffusion.

MODEL COMPOUNDS AND MIXTURES

The model compounds were chosen to cover a range of volatility and polarity from the higher molecular weight polycyclic aromatic compounds (HMWPAC) and included the following: chrysene (mw=228), coronene (mw=300), decacyclene (mw=450), benzanthrone (mw= 230), 1-nitropyrene (mw= 247), and dibenzo[a,i]carbazole (mw=267). A standard

solution containing each of the model compounds at an approximate concentration of 50 ng/μl in benzene was prepared. The solution was stored in the dark and refrigerated until used.

A second model compound mixture chosen to cover a relatively wide molecular weight range of polycyclic aromatic hydrocarbons (PAH) included the following: naphthalene, fluorene, phenanthrene, pyrene, chrysene, and benzo[e]pyrene.

A complex polycyclic aromatic compound (PAC) material was also utilized which consisted of coal tar containing predominately parent ring structures ranging from two to six rings with lower concentrations of alkylated homologs (10) mixed with the PAH fraction (11) of a coal liquid containing relatively higher concentrations of the alkylated parent species.

Adsorbent Spiking

The adsorbents were spiked at 50 ppm with the HMWPAC by injecting 1.0 mL (or 0.5 mL for the PUF) liquid aliquots of the dilute standard solution onto one-gram (0.5 g for the PUF) quantities of the adsorbents. The solvent was allowed to evaporate at room temperature to adsorb the analytes to the adsorbents. Two-gram quantities of the Urban Dust were used for the particle extractions.

The PAH standard mixture and the complex PAC material were spiked on deactivated glass beads at approximately 25 ppm. The glass beads (45-150 μm i.d.) were deactivated by refluxing in a 1:1 mixture of chlorotrimethylsilane and hexamethyldisilazane for 16 hours. Small quantities (100 mg) of the XAD-2 resin were also spiked with approximately 10 ng of each component of the PAH mixture (0.1 ppm).

FLUIDS AND SOLVENTS

Supercritical fluid grade carbon dioxide, an 18.2 wt. % methanol in supercritical fluid grade carbon dioxide mixture, and 99% pure CP grade isobutane (Scott Specialty Gases, Plumsteadville, PA) were used for supercritical fluid extracting solvents. The liquid solvents used for Soxhlet extraction (methylene chloride) and a fluid modifier (methanol) were pesticide grade (Burdick and Jackson).

SECTION 4

EXPERIMENTAL PROCEDURES

OFF-LINE SUPERCRITICAL FLUID EXTRACTION

The adsorbent materials that had been spiked with the model compounds and the air particle sample were subjected to off-line supercritical fluid extraction using carbon dioxide, isobutane, and a methanol-modified (18.2 mole %) carbon dioxide mixture.

Apparatus

A schematic diagram of the instrumentation designed for analytical SFE is shown in Figure 1. A modified Rabbit HPX solvent delivery system equipped with a 10 mL/min pump head and an electronic pressure monitor (Rainin Instrument Co., Woburn, MA) was used to pressurize and deliver the extracting fluids. The pumphead, check valves, and several inches of the inlet line immediately preceding the pumphead were cooled by circulating a -15°C ethylene glycol-water mixture through copper blocks that were machined to fit the pumphead geometry. The inlet check valve cartridge was also modified for direct connection to 1/8 inch stainless steel tubing to allow a larger supply of low pressure solvent to enter the pump. No other modifications to the check valves or piston assembly were required.

The pressurized fluid was transferred to the 316 stainless steel high-pressure extraction vessel (shown in Figure 2) through 1/16 inch stainless steel tubing. The extraction cell was constructed to provide safe operation at pressures over 400 bar and temperatures greater than 200°C. The extraction cell body and top were sealed with double Teflon® O-rings. The extraction vessel was maintained at elevated temperatures in a Hewlett-Packard 5700 gas chromatograph oven. The oven was continuously purged with nitrogen when using flammable extracting fluids or fluid mixtures. The transfer line from the pump extended to the bottom of the extraction cell chamber (~ 5 mL volume) where the sample was placed in a 5.4 x 1.3 cm o.d. borosilicate glass test tube. This design allowed the extraction fluid to move through the sample from bottom to top and then exit the extraction vessel. A stainless steel frit (2.0 to 5.0 μm pore size) was placed in the exit port of the extraction vessel to prevent sample from being flushed out in case of sudden pressure surges. The extract stream was transported from the extraction cell to

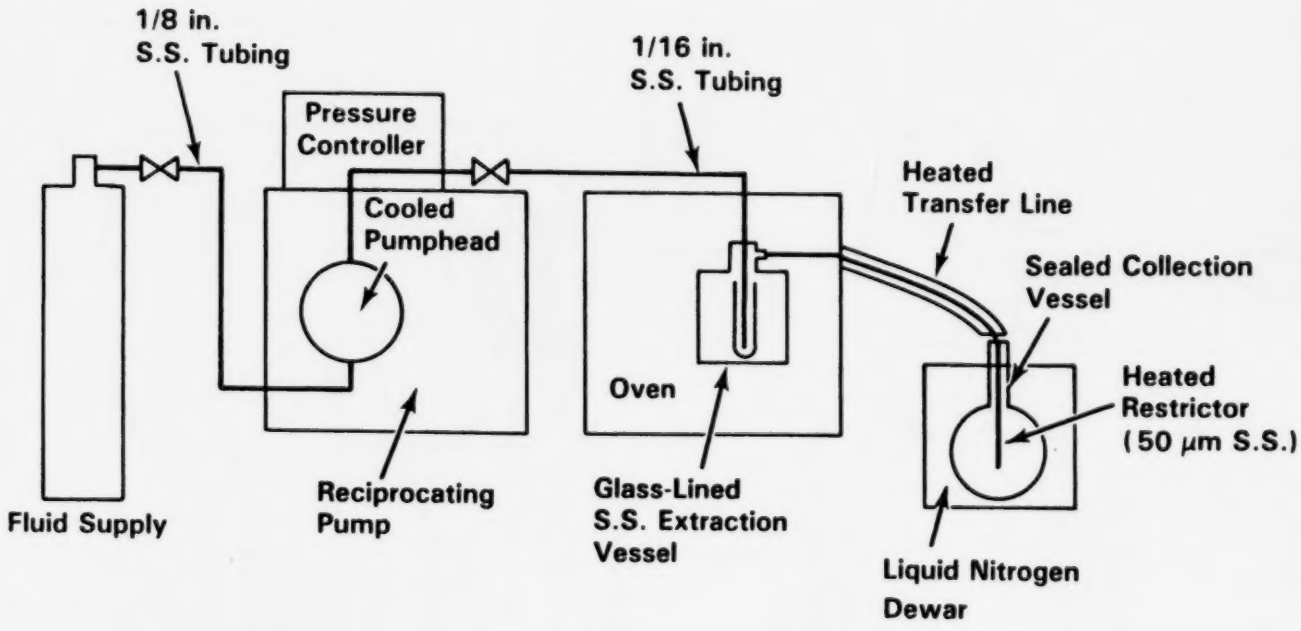

Figure 1. Schematic diagram of the off-line supercritical fluid extraction apparatus.

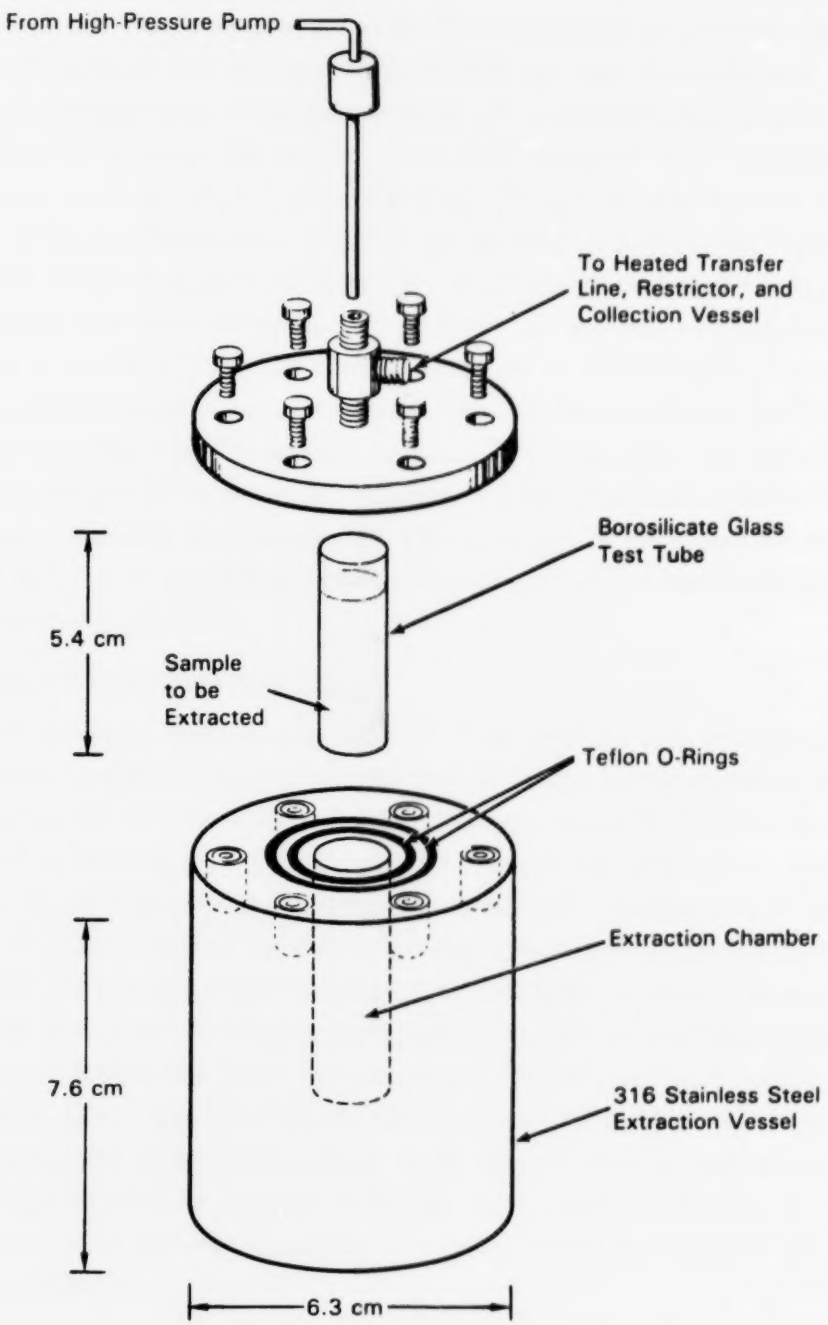

Figure 2. Design of the supercritical fluid extraction vessel.

the exterior of the oven through a heated transfer line containing 1/16 inch i.d. glass-lined stainless steel tubing. In early experiments, the transfer line was connected to a 10-12 cm length of 50 μm i.d. stainless steel tubing which was crimped at the exit end to control the fluid flow rate and serve as a depressurization zone for the extraction stream. Short lengths of fused silica capillary tubing were used in later experiments to provide more reproducible flow characteristics.

The extraction effluents were collected in a sealed round bottom flask cooled in a liquid nitrogen bath. When using pure carbon dioxide, the 50 μm stainless steel restrictor tubing was heated by applying an electrical current (a copper wire was attached near the tip of the stainless steel tubing to provide an electrical contact) to elevate the fluid temperature to prevent the restrictor (positioned inside of the cooled vessel) from freezing closed during the fluid expansion process. The enthalpy of the flowing fluid was adequate with the other fluid systems to maintain the minimum temperature necessary for formation of a two-phase (gas-solid for CO_2) product upon expansion to atmospheric pressure (12). Open collection in which the restrictor was placed through the neck and into the bulb of a 100 mL volumetric flask that was cooled to 0°C was also employed for some experiments.

Supercritical Fluid Extraction Procedures

Extractions with the carbon dioxide and the 18.2 % methanol-carbon dioxide mixture were conducted at temperatures ranging between 125° to 150°C and at a pressure of ~400 bar. Isobutane extractions were conducted in a similar manner at 150°C and 185 bar. The transfer line region was maintained at approximately the same temperature as the oven. The fluid was delivered to the extraction cell by pressurizing at ambient tank pressure (c.a. 60 bar for carbon dioxide and 3 bar for isobutane) and then ballistically pumping to the operating pressure. Fluid compressibility and pumping constraints generally limited the average rate of pressure increase to approximately 20 bar/min. Although pressure control was maintained by duty cycling the pump, overall pressure stability of a few bar was achieved. The flow rates for the supercritical fluids ranged between 4 and 6 mL/min of liquid (~4-6 liters/min gas) and extractions were conducted for times ranging from 20 minutes to an hour. Some variability existed in adjusting the flow rate due to the imprecise nature of crimping the stainless steel capillary and forming the restrictor, but exact amounts of fluid used were obtainable by weighing the frozen or liquefied material trapped in the collection flasks.

Soxhlet Extractions

All supercritical fluid extracted samples were also subjected to Soxhlet extraction. Some samples were Soxhlet extracted prior to SFE to provide a comparison between the two

techniques. Soxhlet extractions were accomplished using acid-washed glassware and previously extracted thimbles with approximately 200 mL of methylene chloride as the extraction solvent. Extractions were conducted for 16-18 hours with a cycle time of about 10 minutes.

Chromatographic Analyses

Capillary gas chromatography (GC) was used to quantify the materials recovered from the supercritical fluid and Soxhlet extractions. For the higher molecular weight materials, which were difficult or impossible to analyze by gas chromatography, capillary supercritical fluid chromatography was utilized. Capillary GC was accomplished with a Hewlett-Packard 5880 gas chromatograph equipped with a capillary injection system, a flame ionization detector, an autosampler, and a 15 m x 0.25 mm i.d. fused silica capillary column coated with crosslinked SE-54 (5% phenyl polymethylphenylsiloxane) stationary phase. The chromatographic conditions included hydrogen carrier gas at a linear velocity of 100 cm/sec and a temperature program starting at 50°C that was ramped to 180°C at 10°C/min followed by a ramp of 4 °C/min to a final temperature of 300°C. Quantification was achieved using an internal standard method with external calibration. 2-Chloroanthracene at a level of 50 ng/µl was used as the internal standard and response factors for each analyte were calculated from three concentration levels of the model compound standards, each of at least 95% purity. NBS SRM 1647, Priority Pollutant PAH, and additional PAH standards of greater than 95 % purity at five concentration levels ranging two orders of magnitude were used as additional calibration standards for the Urban Dust sample analyses.

Quantitative capillary supercritical fluid chromatography was accomplished with a supercritical pentane mobile phase, a 5 m x 50 µm i.d. fused silica capillary column coated with a highly crosslinked SE-54 stationary phase, and fluorescence detection (9). Chromatographic conditions included an operating temperature of 210°C and a pressure program starting at an initial pressure of 27 bar ramped at 0.8 bar/min beginning 10 minutes after injection. Quantification was achieved using an external calibration method with response factors for each quantified compound calculated from four concentration levels of the standard.

ON-LINE SUPERCRITICAL FLUID EXTRACTION-GAS CHROMATOGRAPHY

The samples utilized in this investigation included a complex polycyclic aromatic compound (PAC) material adsorbed on deactivated glass beads, a polycyclic aromatic hydrocarbon standard mixture adsorbed on deactivated glass beads and XAD-2 resin, and a National Bureau of Standards air particulate sample, Urban Dust SRM 1649.

Apparatus

The automated on-line SFE-gas chromatography instrumentation consisted primarily of four sections which included a high pressure pump and extraction cell, a switching valve and interface region, a gas chromatograph with a flame ionization detector, and a minicomputer and associated interface circuitry. A schematic diagram of this instrumentation is shown in Figure 3.

A modified Varian 8500 syringe pump provided a high pressure supply of carbon dioxide to the extraction cell. The carbon dioxide was purified by distilling through activated charcoal while filling the syringe pump. The micro-extraction cells (see Figure 4) were constructed from Swagelok® stainless steel zero volume 1/4" to 1/16" column end fittings (SS-400-6-1ZV) containing two 1/4" o.d. sintered stainless steel frits with 2.0 µm mean pore size separated by a 1/8" long x 1/4 " o.d. x 3/32" i.d. stainless steel insert. The 1/4" o.d. inlet to the extraction cell was made by cutting and silver soldering the smoothed end from standard 1/16" o.d. stainless steel tubing that was inserted through a short length (1-2") of 1/4" o.d. x 5/64" i.d. stainless steel tubing. This design provided an entirely stainless steel extraction cell with a total volume of approximately 15 µl (excluding internal frit volumes). Larger cell volumes could be obtained by using larger i.d. and/or longer inserts. The extraction cell and several inches of inlet tubing were placed inside a thermostatically regulated heating block to control the fluid and extraction cell temperature. Extraction temperatures in the range of 40-50°C were generally used.

An air-actuated Rheodyne 7010 six-port switching valve was used to direct the extraction cell effluent to either an exterior collection reservoir or to the gas chromatographic column for on-column deposition and concentration of the extract. Short lengths (2-4 cm) of nominally 5 µm i.d. fused silica were used as depressurization restrictors and to maintain supercritical pressures up to the point of on-column deposition or sample collection. The decompressed gas flow rate of the extraction fluid was typically 5 mL/min (~5 µl/min fluid flow). The restrictor for the on-column deposition was mounted through a 1/16" tee to allow the gas chromatograph carrier gas to enter coaxially along the restrictor. This connection was also mounted in a heated block to control the temperature of the restrictor and expansion region. Typically, this region was maintained near the upper operating temperature of the chromatographic oven which was between 250 and 280°C. High pressure carrier gas (300 psi) was also connected to the six-port valve which was alternately switched with the extraction effluent between the chromatographic column and the collection vent to purge any remaining extract from the system.

A Hewlett-Packard 5890 gas chromatgraph equipped with a single flame ionization detector was used to perform chromatographic analyses. Typically, 15 m x 0.25 mm i.d. fused silica capillary columns coated with a 0.25 µm film thickness of crosslinked 5% phenyl polymethylphenylsiloxane (SE-54) were utilized. A short retention gap of deactivated fused

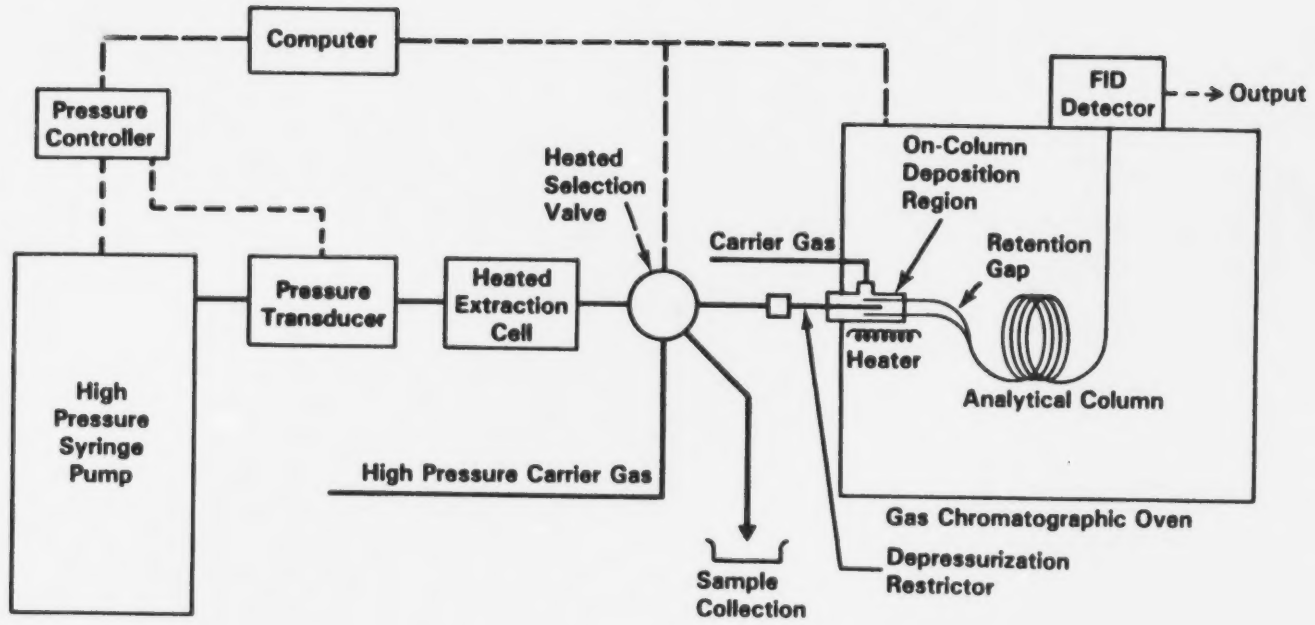

Figure 3. Schematic illustration of the on-line supercritical fluid extraction-gas chromatography instrumentation.

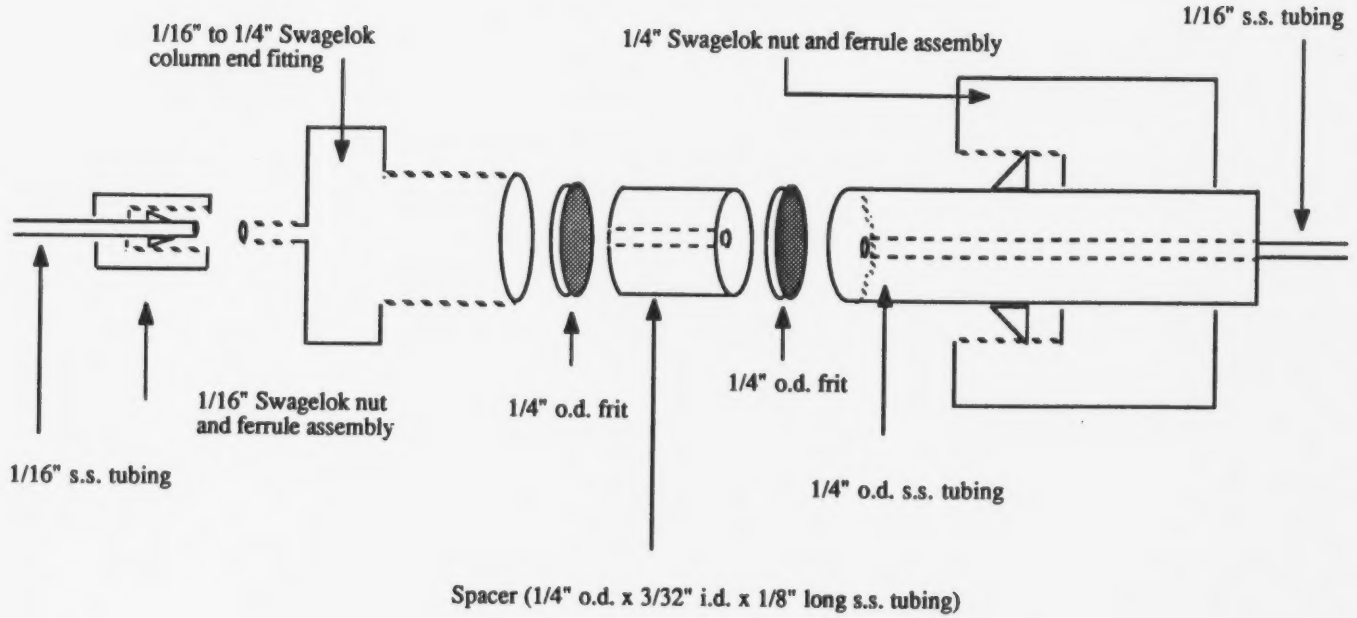
1/16" to 1/4" Swagelok
column end fitting
1/4" Swagelok nut and ferrule assembly
1/16" s.s. tubing
1/16" Swagelok nut
and ferrule assembly
1/4" o.d. frit
1/4" o.d. frit
1/16" s.s. tubing
1/4" o.d. s.s. tubing
Spacer (1/4" o.d. x 3/32" i.d. x 1/8" long s.s. tubing)

Figure 4. Schematic illustration of the micro-extraction cells.

silica tubing (30 cm x 0.53 mm i.d.) was connected to the inlet of the chromatographic column to aid solute focusing and concentration of the extraction effluent. The fused silica retention gap was deactivated by simple silylation with hexamethyldisilazane at 350°C. An all polyimide connection was made between the retention gap and the analytical column to prevent thermal drag during temperature programming. The chromatographic oven temperature was cooled to 30°C or lower during on-column deposition and concentration of the extraction effluent. Subsequent analyses were performed by temperature programming at 4° C/min to 265-280°C. Helium was used for the carrier gas at linear velocities of approximately 40 cm/sec. Detector sensitivity was adjusted to give full-scale peak response for approximately 5 ng per component. Signal output was acquired on a strip chart recorder or with a Nelson Analytical 4416 chromatographic data system.

The instrumentation was automated using an Apple IIe minicomputer with an Adalab interface card (Interactive Microware Inc, State College, PA) and other in-house designed control circuitry. System automation included computer operation of the syringe pump to control pressure, switching of the six-port valve, start-up of the gas chromatograph temperature program cycle, and initiation and termination of data acquisition. A Forth computer program allowed automated operation in which a sample could be subjected to numerous extractions at selected pressures for defined time intervals. In addition, the computer checked if fluid was still available in the pump, if the correct extraction pressure was reached, and if the oven temperature was ready for another cycle to begin.

Automated Extractions

For the qualitative extraction-analyses, approximately 10 mg of the various sample matrices were loaded into the extraction cell. The system was automated to allow selective extractions by extracting at different pressures and analyzing each extract. Typically, extractions would be started at lower pressures and then progressively increased for each additional extraction. The extractions were generally initiated at pressures near the critical pressure (73 bar) of carbon dioxide with the effluent being collected on-column for 2-5 minutes. The effluent would then be vented, while a high pressure carrier gas purged the restrictor during the gas chromatographic analysis. After an analysis was completed, the supercritical fluid extraction pressure would be increased and another analysis cycle performed.

In the quantitative extraction-analysis mode, an exhaustive extraction was performed on the matrix at a single pressure with the entire extraction effluent being deposited in the chromatographic column in a single step. The detector was calibrated by injecting standards of known concentration bracketing the expected levels of the spiked components. These injections were made utilizing a standard splitless injection mode.

DIRECT SUPERCRITICAL FLUID EXTRACTION-MASS SPECTROMETRY

Small quantities (~10 -20 mg) of the adsorbents that were spiked at 50 ppm with the model compounds were subjected to extraction using carbon dioxide, isobutane, and methanol (18.2 mole %) modified carbon dioxide fluid systems. The extraction effluent was monitored in real-time by mass spectrometry.

Apparatus and Conditions

The instrumentation used for direct supercritical fluid extraction-mass spectrometry (SFE-MS) is similar to that previously described (6,7). A schematic illustration of this instrumentation is shown in Figure 5. High pressure extraction fluid was generated using a Varian 8500 syringe pump. The pump was modified to operate under computer control to allow pressure programming capability. Initial extraction pressures ranging from 114 to 135 bar with ramp rates of either 10 or 20 bar/min and final pressures ranging from 195 to 400 bar were used for the different fluid systems. A Hewlett-Packard 5790 gas chromatograph oven provided constant temperature conditions for the extraction vessel and heated the air used to control the temperature of the mass spectrometer interface probe. Operating temperatures ranged between 50° and 150°C depending on the fluid system.

The same type of extraction cell as used in the SFE-GC studies (Figure 4) was used for these extractions. The effluent from the extraction cell was transported to the ion source through 50 µm i.d. deactivated fused silica tubing. A short length (2-3 cm) of nominally 5 µm i.d. fused silica tubing served as a restrictor to allow depressurization of the extraction effluent into the chemical ionization (CI) source of an Extranuclear quadrupole mass spectrometer. The interface included provisions to heat the restrictor tip to prevent condensation of the fluid and/or analytes during depressurization (12) and temperatures greater than 350°C were utilized (it should be noted that the actual fluid temperature probably never exceeds that of the pre-expansion fluid). Isobutane was used as the CI reagent gas for the carbon dioxide extractions and the isobutane and methanol from the respective extraction fluids provided the necessary chemical ionization for optimum performance. The mass spectrometer was adjusted to scan the expected $(M+1)^+$ ions for each of the model compounds. The extractions were conducted until the signal intensities returned to near baseline conditions which usually required between twenty to sixty minutes.

QUANTITATIVE FLUORESCENCE DETECTION SUPERCRITICAL FLUID CHROMATOGRAPHY

The quantitative analysis capability was evaluated for a fluorescence detection supercritical fluid chromatography (SFC) system used to obtain high detection sensitivity with fluid systems that are incompatible with flame ionization.

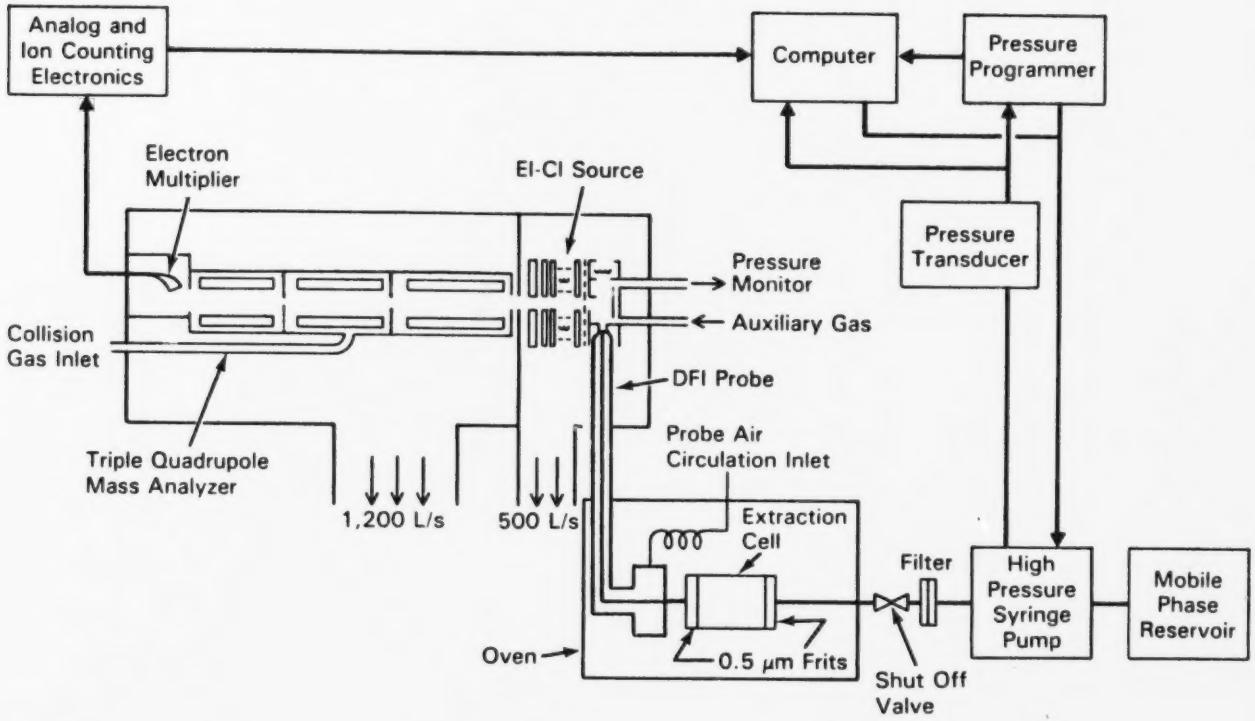

Figure 5. Schematic diagram of the direct supercritical fluid extraction-mass spectrometry instrumentation.

Apparatus

Essentially the same instrumentation as used for flame ionization detection SFC (13,14) was modified for fluorescence detection. The high pressure supply of the chromatographic mobile phase was controlled and delivered by a Brownlee MPLC dual syringe pump. The pump was operated in the SFC mode which allowed pressure-controlled operation and pressure programming capability. A Hewlett-Packard 5840 gas chromatograph oven was used to provide constant temperature conditions. A Valco C14W HPLC valve with a 60 nl volume rotor was used for sample introduction. The injection valve was maintained at ambient temperatures and was connected to the chromatographic column through a splitter. The splitter was adjusted to allow approximately a 1:5 flow into the chromatographic column. The chromatographic column was typically a 5 m length of 50 μm i.d. fused silica that was deactivated and coated with a 0.25 μm film of a 50% phenyl polymethylphenylsiloxane stationary phase (SE-54) that had been subjected to extensive *in-situ* crosslinking (15).

Detection was accomplished using a modified Schoeffel Instruments FS 970 HPLC fluorescence detector (9). The original flow cell was removed and replaced with a length of 250 μm i.d. fused silica capillary with a small window formed by burning off 3-4 mm of the polyimide coating. The analytical column (210 μm o.d.) was inserted through the larger tubing and extended to a point immediately below the window. The larger window tubing and the analytical column were sealed with a Supelco butt-connector. Decompression of the supercritical mobile phase and flow rate regulation were accomplished by connecting a short length of restrictor tubing to the exit end of the window tubing. Consequently, detection was accomplished prior to decompression, but since the detector region was unheated, the fluid was in the liquid state. The small window on the 250 μm i.d. window tubing was carefully aligned to the light path of the tungsten lamp. Appropriate slits were used to illuminate only the interior of the cell to minimize the background from diffracted light.

Supercritical Fluid Chromatography

The above instrumentation was used to analyze the HMWPAC model compound mixture and actual extracts from the off-line extraction studies. Pentane at a temperature of 210°C was used for the mobile phase with typical pressure programs beginning and holding at 27 bar for ten minutes followed by a ramp of 0.8 bar/min to a final pressure of approximately 55 bar. The linear velocity of the mobile phase was adjusted to approximately 3 cm/sec. A detector excitation wavelength of 270 nm was utilized and all emissions greater than 270 nm were collected.

Quantitative analysis was achieved by multi-level external calibration. Standard mixtures of the model compounds with concentrations bracketing the expected analysis concentration were

analyzed. Multiple chromatographic analyses were conducted for both the calibration procedures and the actual analyses of the extracts. The signal output of the fluorescence detector was acquired and processed with a Nelson 4416 chromatographic data system.

SOLUTE FOCUSING IN SUPERCRITICAL FLUID CHROMATOGRAPHY

The development of a method for the focusing of solutes at the head of a chromatographic column in supercritical fluid chromatography is essential for the implementation of splitless injection methods and for efficient on-line supercritical fluid extraction-supercritical fluid chromatography. The primary approach utilized in this investigation involved thermal focusing in which temporary density gradients were created in the mobile phase through localized manipulations of temperature.

Apparatus

A flame ionization detection capillary supercritical fluid chromatograph (13,14) was used for these studies. This instrumentation utilized a modified Varian 8500 syringe pump to deliver high pressure carbon dioxide for the chromatographic mobile phase. The pump was computer driven to allow pressure controlled operation with density programming capability. A Hewlett-Packard 5700 gas chromatograph was used to thermostat the chromatographic column and provide flame ionization detection. Operating temperatures ranging between 40° and 100°C were utilized. A 10 m x 50 μm i.d. fused silica column coated with a crosslinked 5% phenyl polymethylphenylsiloxane (SE-54) stationary phase was used for the analytical column. Split injection was obtained using a Valco C14W HPLC injection valve with a 60 nl rotor volume. The injection valve was mounted outside the oven and connected to the chromatographic column through a flow splitter that allowed approximately a 1:5-1:20 flow into the chromatographic column. Splitless injection was obtained by directly connecting the chromatographic column to the injection valve with a Valco 1/16" to 1/32" zero dead volume reducing connection. Prior to detection, the supercritical fluid mobile phase was decompressed and the linear velocity controlled to between 1 and 2 cm/sec by connecting the terminal end of the chromatographic column to a short length (~25 mm) of 5 μm i.d. fused silica restrictor tubing.

Thermal focusing was accomplished by heating various 8 cm lengths of the first 50 cm of the chromatographic column to temperatures ranging between 75° and 220°C. This localized heating was expected to create sharp density drops in the chromatographic mobile phase. The fused silica column was convectively heated by correctly positioning it in a length of tight-fitting stainless steel capillary tubing and applying electrical current. The temperature was monitored with a thermocouple. Heat was applied for various time periods following injection and prior to density programming. A standard alkane mixture was used to evaluate the methodology.

SECTION 5

RESULTS AND DISCUSSION

OFF-LINE SUPERCRITICAL FLUID EXTRACTION

The potential advantages of SFE make this approach worthy of investigation for analytical applications. In addition to the possibility for higher extraction efficiencies from difficult matrices, this methodology also provides the potential for faster extraction rates and the ready capability for automated, on-line interfacing to chemical analysis methods. Since many supercritical fluid solvents are gases at ambient conditions, additional advantages accrue from the ease of solvent removal and concentration of the extract. The approach taken in this investigation was to evaluate and compare the overall extraction efficiencies of the supercritical fluid methods by collecting the extraction effluents and using off-line analysis methods. Several higher molecular weight compounds spanning a range of polarity were chosen to present a reasonable challenge for extraction from a variety of adsorbent matrices.

Fluid Systems and Instrumentation

A summary of the critical properties of the fluid systems and the extraction parameters employed in this investigation is given in Table 1. In addition to the pure fluid systems of carbon dioxide and isobutane, a more polar binary fluid system utilizing a carbon dioxide-methanol mixture was also employed. The addition of 20 mole percent methanol to carbon dioxide significantly increased the critical parameters for the resulting binary fluid system (16), but only slightly increased the density (17). The increased solvent power for this mixture compared to the pure fluid system is reflected by the approximately thirty percent larger Hildebrand solubility parameter (18) and other specific H-bonding interactions. All solubility parameters were estimated based on the actual fluid densities utilized for the extractions. The solubility parameter for the binary mixture was calculated assuming that the contributions were additive and proportional to composition. Extraction temperatures were chosen to be at least 10°C above the critical temperatures of the fluids and to be somewhat similar to minimize any temperature effects on adsorbate desorption from the matrices. Carbon dioxide and isobutane offer advantages in a practical sense since they are gaseous under ambient conditions and do not require evaporation

TABLE 1. SUMMARY OF FLUID PROPERTIES AND EXTRACTION PARAMETERS

Fluid	Critical Temperature (°C)	Critical Pressure (bar)	Extraction Conditions			
			Temp (°C)	Pressure (bar)	Density (g/mL)	Solubility Parameter (δ)
CO_2	31	73	125	400	0.68	6.0
Isobutane	135	37	150	185	0.45	5.8
CO_2-CH_3OH[a]	120	167	130	400	~0.72[b]	7.7[c]

[a]20 mole percent methanol mixture; critical parameters from ref. 16.

[b]Estimated using methods described in ref. 17.

[c]Does not include H-bonding contributions.

thereby eliminating the potential loss of the more volatile components during concentration and sample work-up. However, the carbon dioxide-methanol fluid mixture has the advantage of the specific solvent-solute interactions due to H-bonding, and for potential deactivation of active adsorbent sites with methanol (desorption by displacement).

A very important consideration in analytical extraction is the methodology for collecting the sample during the depressurization process. Depending upon the exact fluid conditions, it is possible for analyte molecules to nucleate, become entrained in the expanding gas and form an aerosol which can be easily lost to the atmosphere. Recent experiments have suggested that solute particles in the 0.01- 0.02 μm size range can be formed by the rapid expansion of supercritical fluid solutions through pressure restrictors (12). The impact of these phenomena is illustrated by the data presented in Table 2 in which different collection methods were utilized for otherwise identical extractions. These data are based on atleast two replicate experiments and the 10 % error range includes variability between experiments and possible uncertainty involved with the GC analyses. One method of collection employed trapping the extraction effluent in the bulb of an open 100 mL volumetric flask that was cooled to 0°C, and the other method used a sealed vessel that was cooled in liquid nitrogen to freeze the extraction effluent. Although the open vessel was cooled and had a relatively small opening through a several centimeter long

TABLE 2. COMPARISON OF EXTRACTION RECOVERIES FROM XAD-2 RESIN[a] USING DIFFERENT COLLECTION METHODS[b]

	% Recovery (± 10 %)	
Compound	Open Collection	Sealed Collection
Chrysene	2.2	75
Benzanthrone	2.5	79
1-Nitropyrene	1.8	83
Dibenzo[a,i]carbazole	6.3	95
Coronene	8.2	81
Rubrene	0	25

[a]Extraction Conditions: CO_2 at 150°C and 410 bar with ~250 mL of liquid.

[b]Open collection in narrow-necked flask at 0°C; Sealed collection in liquid nitrogen cooled flask.

neck, significant losses of the analyte compounds occurred. Since the volatility of these compounds at 0°C was very low, the most likely mechanism for loss was through solute aerosol formation. By freezing the extraction effluent in a sealed vessel any possibility of sample loss through either volatility or aerosol formation was completely eliminated. This collection method was adopted for these studies to ensure that collection phenomena did not interfere with supercritical fluid extraction evaluation.

The effects of aerosol formation on sample loss during collection is further illustrated with the data in Table 3 in which different extraction conditions employing open collection were utilized. Again, these data are based on atleast two replicate experiments and the 10 % error range includes variability between experiments and possible uncertainty involved with the GC analyses. Previous work has shown that at 150°C the expansion of carbon dioxide produces only a single gas phase, whereas expansion at 50°C is expected to yield a two-phase (gas-solid) CO_2 product. At the higher temperature the nucleation mode producing very small solute particles (<0.2 μm) dominates and low collection efficiency might be expected since these particles will settle very slowly and be easily entrained in the CO_2 gas flow out of the collection vessel. At lower temperatures the solute will associate to a significant extent with the much larger frozen carbon dioxide particles and have high deposition and collection efficiencies. Interestingly,

TABLE 3. COMPARISON OF EXTRACTION RECOVERIES FROM XAD-2 USING OPEN COLLECTION AND VARIOUS EXTRACTION CONDITIONS[a]

Compound	% Recovery (± 10%)		
	CO_2 (150°C)	CO_2 (50°C)	CO_2-CH_3OH[b] (150°C)
Chrysene	2.2	25	60
Benzanthrone	2.5	28	62
1-Nitropyrene	1.8	24	29
Dibenzo[a,i]carbazole	6.3	10	65
Coronene	8.2	6.5	62

[a]Extractions were conducted at 410 bar with 250 mL of liquid.

[b]20 mole percent methanol mixture.

a molecular weight increased, recoveries appeared to decrease at the lower temperature. Although still uncertain, this apparent decrease in recovery probably reflects lower extraction efficiency at 50°C for the higher molecular weight compounds due to lower solubility and the limited solvent volume utilized. The use of a polar modifier with a high critical temperature, such as methanol, in carbon dioxide also substantially decreased the aerosol loss process, as reflected by the much higher extraction recoveries. Since these recoveries, shown in Table 3, were all lower than that obtained by the sealed collection method (see Table 2), the higher recoveries can be attributed to improved collection, rather than to more efficient extraction. The enhanced collection efficiency for the CO_2-methanol solvent can be ascribed to the formation of sizable liquid methanol droplets during expansion, which likely contain or scavenge many of the analyte molecules, and have larger deposition efficiencies due to their size and liquid character. Consequently, it may be possible to use specialized extraction conditions to obtain efficient collection using simplified open collection methods (as opposed to sealed collection) based upon mixed fluid systems.

Supercritical Fluid Extraction of Adsorbents

The spiked adsorbents were subjected to SFE using the various fluid sytems. After SFE, the same adsorbents were Soxhlet extracted to determine if any additional material could be

recovered. The opposite process was also carried out using spiked adsorbents that had been previously Soxhlet extracted to determine if additional material could be recovered by SFE. To check the reproducibility of the spiking, extraction, and analysis methods, numerous (e.g., ten) XAD-2 resin adsorbents were subjected to independent Soxhlet and supercritical carbon dioxide extraction. Variability in extraction recoveries ranged from less than 5% to approximately 15% and appeared to be distributed randomly. Since the overall variability between Soxhlet and SFE was similar, the greatest contribution to the variance can probably be attributed to inhomogeneous spiking. SFE removed a significant quantity of background material from some of the adsorbents, but in most cases the extracted contaminants did not interfere with the GC analyses. The data listed in Tables 4-6 are based on multiple replicate experiments (usually greater than five) and in general, the 10 % error range reflects differences between replicate experiments and possible uncertainty involved in the GC analyses.

Extraction data for spiked XAD-2 resin using Soxhlet extraction and various supercritical fluid systems are listed in Table 4. The Soxhlet extraction entries under the various supercritical fluid system headings refer to Soxhlet extractions conducted after the samples were first subjected to SFE. High recoveries (approaching quantitative) of all the model compounds were achieved using Soxhlet extraction. Similar recoveries were obtained for the lower molecular weight compounds with SFE, but as the molecular weight increased, progressively lower recoveries were obtained with carbon dioxide. This behavior is particularly evident for the highest molecular weight compound, decacyclene. Subsequent Soxhlet extraction did not recover any additional lower molecular weight material, but it did recover corresponding increased quantities of the higher molecular weight analytes. This behavior is explained by the lower solubility of the higher molecular weight analytes in the nonpolar carbon dioxide fluid system. Extraction with a larger volume of fluid would have improved the extraction efficiency. In fact, the extractions described in Table 2 utilized somewhat larger fluid volumes and improved recoveries for the higher molecular weight components were observed. However, for components with low solubility in carbon dioxide, a better approach is to utilize a stronger solvating fluid.

SFE with isobutane provided better overall extraction efficiency than carbon dioxide and only low quantities of material, if any, were recovered by subsequent Soxhlet extraction. It would be expected that the higher molecular weight polycyclic aromatic compounds would have greater solubility in isobutane than in carbon dioxide. Similar improved extraction recoveries over the complete model compound range were also obtained with the methanol modified carbon dioxide and only traces of material were recovered by subsequent Soxhlet extraction. Again, this is consistent with the higher solvating power of this fluid system. It is important to note that these

TABLE 4. EXTRACTION COMPARISON OF XAD-2 RESIN USING SOXHLET AND VARIOUS SFE FLUID SYSTEMS

	% Recovery (± 10 %)						
		CO_2[b]		Isobutane[d]		CO_2-CH_3OH[e]	
Compound	Soxhlet Only[a]	SFE	Soxhlet[c]	SFE	Soxhlet[c]	SFE	Soxhlet[c]
Chrysene	79	84	0	86	5	88	3
Benzanthrone	86	98	0	110	0	88	0
1-Nitropyrene	93	81	0	70	0	98	0
Dibenzo[a,i]carbazole	88	54	22	96	6	97	0
Coronene	81	46	63	93	9	90	5
Decacyclene	85	6	88	--	--	--	--

Conditions:

[a] Methylene chloride for 16 hours.

[b] CO_2 at 125°C and 400 bar with ~ 200 mL of liquid.

[c] Extraction of same sample after SFE.

[d] Isobutane at 150°C and 185 bar with ~ 300 mL of liquid.

[e] 20 mole % methanol in carbon dioxide at 130°C and 400 bar with ~ 210 mL of liquid.

supercritical extractions were accomplished in approximately thirty to forty-five minutes compared to over sixteen hours for the Soxhlet extractions and that fluid solubility rather than desorption rates appear to provide the major limitations in this work. These results suggest more rapid pumping of fluid through the extraction cell should allow a substantial increase in speed.

SFE of XAD-2 resin that had been previously Soxhlet extracted recovered only low quantities (2-5%) of coronene and decacyclene. No significant differences were observed for the three fluid systems. In most cases it appears that Soxhlet extraction recovered essentially all of the analytes and only trace amounts were still available for SFE.

Extraction comparison data for the spiked polyurethane foam (PUF) using Soxhlet and SFE are listed in Table 5. Essentially the same trends as were observed for the XAD-2 resin were also displayed by this adsorbent. Soxhlet extraction provided nearly quantitative recoveries with little or no discrimination as a function of molecular weight or polarity. Supercritical carbon dioxide quantitatively recovered the lower molecular weight compounds and only small quantities of the higher molecular weight materials (with the exception of decacyclene) could be recovered by subsequent Soxhlet extraction. In fact, the absolute quantities of the higher molecular weight analytes that were recovered by SFE were essentially identical to those obtained by Soxhlet extraction. The decacyclene, however, displayed very low extractability with supercritical carbon dioxide, which can probably be attributed to very low solubility in the fluid. High extraction recoveries were also obtained with isobutane, but lower extractability of the more polar compounds (nitropyrene and dibenzocarbazole) was observed. These components were recovered with subsequent Soxhlet extraction. SFE using the more polar methanol modified carbon dioxide recovered all of the materials with high yields except for the nitropyrene. Subsequent Soxhlet extraction, however, failed to recover any of this compound which suggests that it was probably not present at the expected level on the adsorbent (possibly due to adsorption on spiking vessel walls) or that it was very tightly bound to the adsorbent.

The supercritical fluid extractions of the PUF were all accomplished using less total fluid than was used for the XAD-2 resins (see Tables 4 and 5 for the exact amounts), but similar or better extraction efficiencies were achieved in most cases. Again, only approximately thirty minutes were required to complete each extraction. Undoubtedly, larger fluid volumes would have led to more complete extraction of the more polar and higher molecular weight components with the carbon dioxide and isobutane fluids. Subsequent SFE of PUF that had been previously Soxhlet extracted did not yield any additional material. It is likely that the Soxhlet extractions recovered essentially all the available material.

TABLE 5. EXTRACTION COMPARISON OF PUF USING SOXHLET AND VARIOUS SFE FLUID SYSTEMS.

	% Recovery (± 10 %)						
		CO_2[b]		Isobutane[d]		CO_2-CH_3OH[e]	
Compound	Soxhlet Only[a]	SFE	Soxhlet[c]	SFE	Soxhlet[c]	SFE	Soxhlet[c]
Chrysene	95	88	0	94	0	97	5
Benzanthrone	86	92	0	101	0	102	0
1-Nitropyrene	82	70	0	54	21	33	3
Dibenzo[a,i]carbazole	76	70	14	51	15	93	7
Coronene	85	82	14	78	6	80	4
Decacyclene	--	6	92	--	--	--	--

Conditions:

[a] Methylene chloride for 16 hours.

[b] CO_2 at 125°C and 400 bar with ~ 163 mL of liquid.

[c] Extraction of same sample after SFE.

[d] Isobutane at 150°C and 185 bar with ~ 125 mL of liquid.

[e] 20 mole % methanol in carbon dioxide at 130°C and 400 bar with ~ 120 mL of liquid.

More background contaminants were extracted from the PUF than from the XAD-2 resin and, as would be expected, the methanol-modified carbon dioxide removed the largest quantities of extraneous compounds. Typical capillary gas chromatograms of PUF and XAD-2 resin SFE extracts obtained with methanol-modified carbon dioxide are shown in Figure 6. Both matrices were Soxhlet extracted prior to spiking with the model compounds. As can be observed, significantly greater quantities of contaminant compounds were extracted from PUF than from XAD-2 resin. Blank extractions of empty extraction cells gave essentially clean extracts for all the fluid systems. Supercritical carbon dioxide extraction was used to clean selected PUF adsorbents prior to spiking with the model compounds. No apparent differences in extractability or appearance (other than being whiter) were observed for these previously extracted adsorbents. Extraction of the PUF with methanol modified carbon dioxide, however, appeared to disrupt the matrix and render the foam somewhat brittle. In fact, the PUF matrix itself appeared to be slightly soluble in this fluid system. Interactions of fluids with polymer phases is known to occur (8), and even to cause physical changes to highly crosslinked matrices which may be attributed in part to forces generated during depressurization.

To provide a more challenging matrix for extraction evaluation, Spherocarb, an adsorbent generally reserved for volatile and semi-volatile lower molecular weight compounds, was also utilized. The Soxhlet and SFE extraction comparison data for this adsorbent are listed in Table 6. Only very low quantities of the less polar model compounds were recovered by Soxhlet extraction. Supercritical carbon dioxide extraction did not recover any appreciable portion of the analytes. Supercritical isobutane and methanol modified carbon dioxide extraction recovered small quantities of the model compounds, and overall removed slightly more material than Soxhlet extraction. All of the extraction methods failed to recover any of the most polar model compound, 1-nitropyrene. Subsequent Soxhlet extraction after SFE recovered trace quantities of chrysene and coronene, but subsequent SFE after Soxhlet extraction did not recover any additional analyte material. Although SFE with the stronger solvating fluid systems provided better extraction efficiency than Soxhlet extraction, none of the methods was able to extract significant quantities of the spiked analytes.

Supercritical Fluid Extraction of Urban Dust

In addition to model systems, SFE was also used to extract Urban Dust (SRM 1649), which is an air particulate matter sample and a National Bureau of Standards certified reference material. In this evaluation, the samples were also extracted by both Soxhlet and supercritical fluid methods. Urban Dust is a very complex matrix and numerous components were extracted using both methods. A sample was first subjected to Soxhlet extraction and then followed by SFE to determine if additional or different components could be removed. The converse was

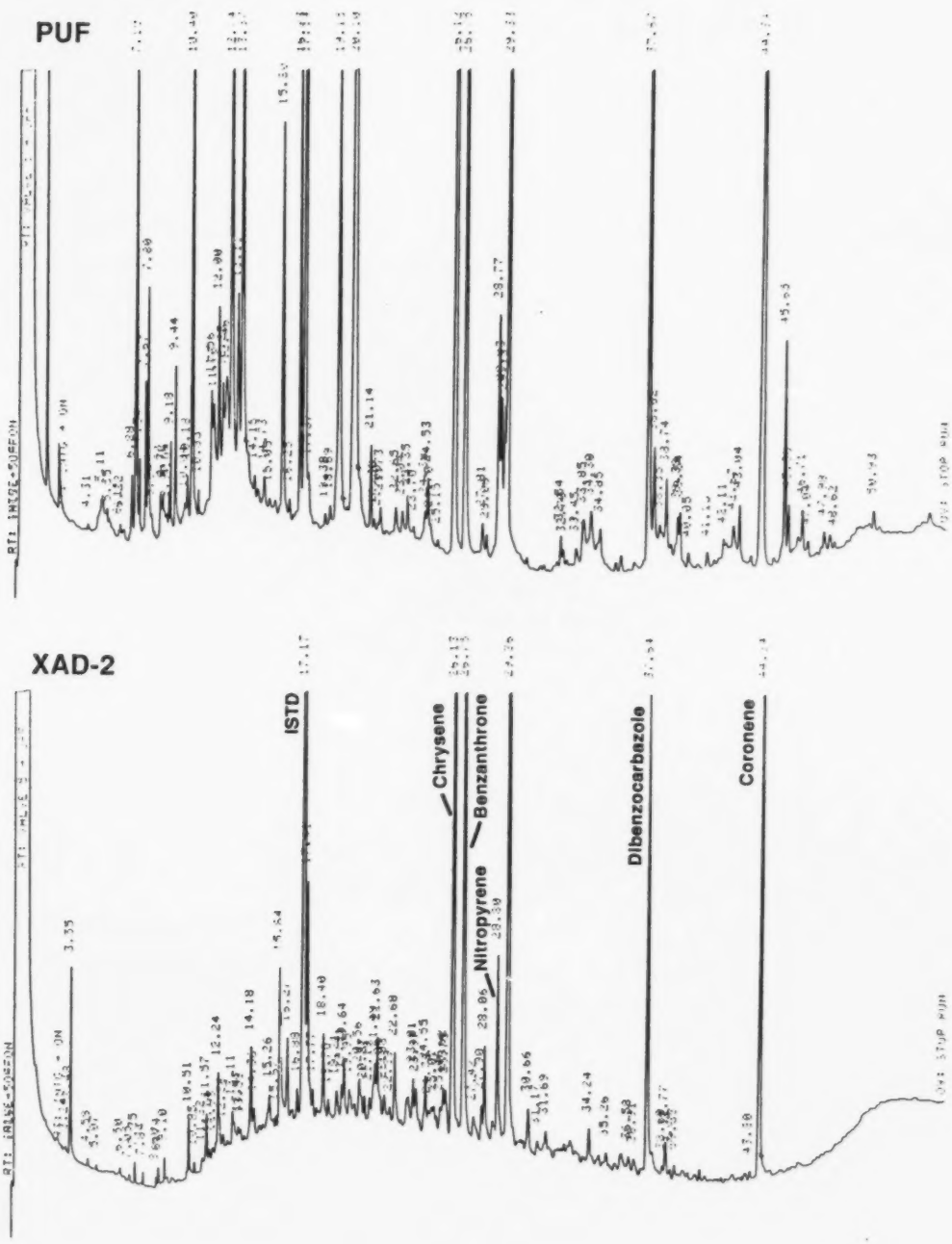

Figure 6. Typical capillary gas chromatograms of PUF and XAD-2 resin extracts obtained with methanol-modified carbon dioxide SFE.

TABLE 6. EXTRACTION COMPARISON OF SPHEROCARB ® USING SOXHLET AND VARIOUS SFE FLUID SYSTEMS

	% Recovery (± 10 %)						
		CO_2[b]		Isobutane[d]		CO_2-CH_3OH[e]	
Compound	Soxhlet Only[a]	SFE	Soxhlet[c]	SFE	Soxhlet[c]	SFE	Soxhlet[c]
Chrysene	5	0	1	2	2	2	0
Benzanthrone	4	0	0	11	0	3	0
1-Nitropyrene	0	0	0	0	0	0	0
Dibenzo[a,i]carbazole	0	0	0	3	2	3	0
Coronene	1	0	2	4	3	3	2

Conditions:

[a] Methylene chloride for 16 hours.
[b] CO_2 at 125°C and 400 bar with ~ 215 mL of liquid.
[c] Extraction of same sample after SFE.
[d] Isobutane at 150°C and 185 bar with ~ 400 mL of liquid.
[e] 20 mole % methanol in carbon dioxide at 130°C and 400 bar with ~ 214 mL of liquid.

also applied in which the sample was first subjected to SFE and then followed by Soxhlet extraction. Unfortunately, several species coeluted during GC analysis which made it impossible to monitor (by GC retention time only) many of the PAH present in the matrix. Extraction comparison data of selected polycyclic aromatic hydrocarbons (PAH) using Soxhlet and supercritical carbon dioxide extraction are listed in Table 7. Approximately 4.9 % (by weight) of the material was extracted with the Soxhlet method and an additional 1.0 % was recovered with subsequent SFE. Even if more overall material (total weight) was recovered with the initial Soxhlet extraction, subsequent SFE generally removed larger quantities of the monitored PAH. In fact, several of these components were only detected in the supercritical fluid extract. When fresh Urban Dust was subjected to SFE first, approximately 5.0 % (by weight) of the material was extracted. Subsequent Soxhlet extraction removed an additional

TABLE 7. EXTRACTION COMPARISON OF URBAN DUST USING SOXHLET AND CARBON DIOXIDE SFE

	Recovery (μg/g, ± 10 %)				
Compound[a]	Sox.[b]	SFE[c] After Sox.	SFE[c]	Sox.[b] After SFE	NBS
Naphthalene	6	<0.01	<0.01	1.7	--
2-Methylnaphthalene	2.7	0.09	<0.01	0.5	--
Dibenzofuran	<0.01	0.02	<0.01	<0.01	--
Fluorene	<0.01	0.07	<0.01	<0.01	--
Dibenzothiophene	0.1	0.02	0.4	<0.01	--
Phenanthrene	<0.01	0.1	<0.01	<0.01	4.5
Fluoranthene	1.2	5.9	2.0	<0.01	7.1 (Cert.)
Pyrene	1.9	2.6	5.9	1.3	6.2-7.2
Chrysene	<0.01	3.1	4.2	<0.01	3.5-3.7
Benzo[e]pyrene	<0.01	0.7	1.3	<0.01	3.3

[a]Compounds identified on the basis of GC retention time only.

[b]Methylene chloride for 16 hrs.

[c]CO_2 at 125°C and 400 bar with ~250 mL of liquid.

1.3 %. Again, larger quantities of the monitored PAH compounds were extracted by SFE. In general, reasonable agreement between the concentrations of the PAH determined from the sum of the Soxhlet and SFE methods and the NBS certified values were obtained. For instance, the certified concentration of fluoranthene is 7.1 ± 0.5 μg/g and values of 7.1 and 2.0 μg/g, Σ(Sox + SFE after Sox) and Σ(SFE + Sox. after SFE), respectively, were obtained in this study. Other NBS values (not certified) include 7.2 ± 0.2 μg/g (GC method) for pyrene (compared to 4.5 and 7.2 μg/g obtained in this study), 3.5 ± 0.1 μg/g (LC1 method) for chrysene (compared to 3.1 and 4.2 μg/g obtained in this study), and 3.3 ± 0.2 μg/g (GC method) for benzo[e]pyrene (compared to 0.7 and 1.3 μg/g obtained in this study). These results are surprising since no sample clean up or multilevel analyses were performed. Capillary GC chromatograms of the Soxhlet extract and the SFE after Soxhlet extract from these analyses are shown in Figure 7. It is interesting to compare the peak profiles and intensities from these extracts and to note that the components in the SFE after Soxhlet extract were better resolved and were generally of higher intensity. (The area of the internal standard in the Soxhlet extract was only 80% as large as the area of the internal standard in the SFE after Soxhlet extract, but this difference does not account for the generally higher peak intensities.)

Similar extraction comparison data for selected PAH utilizing isobutane as the supercritical fluid are listed in Table 8. Again, numerous components were extracted by both the Soxhlet and SFE methods and since no PAH isolation steps were utilized during sample preparation, coeluting species prevented quantification of many of the PAH. Approximately 6.4 % (by weight) of the Urban Dust was Soxhlet extractable and only an additional 0.4 % of material was recovered by subsequent SFE. However, additional quantities of PAH were still recovered by SFE. When SFE was applied first, approximately 2.8 % of the material was initially extracted and subsequent Soxhlet extraction recovered only 0.9 % additional material. The SFE method essentially recovered all of the monitored components since none were detected with subsequent Soxhlet extraction. Comparison of the concentration levels of selected components from these extractions to the NBS values are also favorable. The certified concentration of benzo[ghi]perylene is 4.5 ± 1.1 μg/g (compared to 3.2 and 1.5 μg/g, Σ(Sox + SFE after Sox) and Σ(SFE + Sox. after SFE), respectively, obtained in these studies) and the NBS value (not certified) for phenanthrene is 4.5 ± 0.3 μg/g (compared to 4.9 and 2.7 μg/g obtained in these studies).

These studies indicate that the PAH from Urban Dust can be extracted using supercritical fluid extraction methods. Although in some cases, greater overall quantities of material (gravimetric determinations) could be obtained by Soxhlet extraction, the SFE methods generally

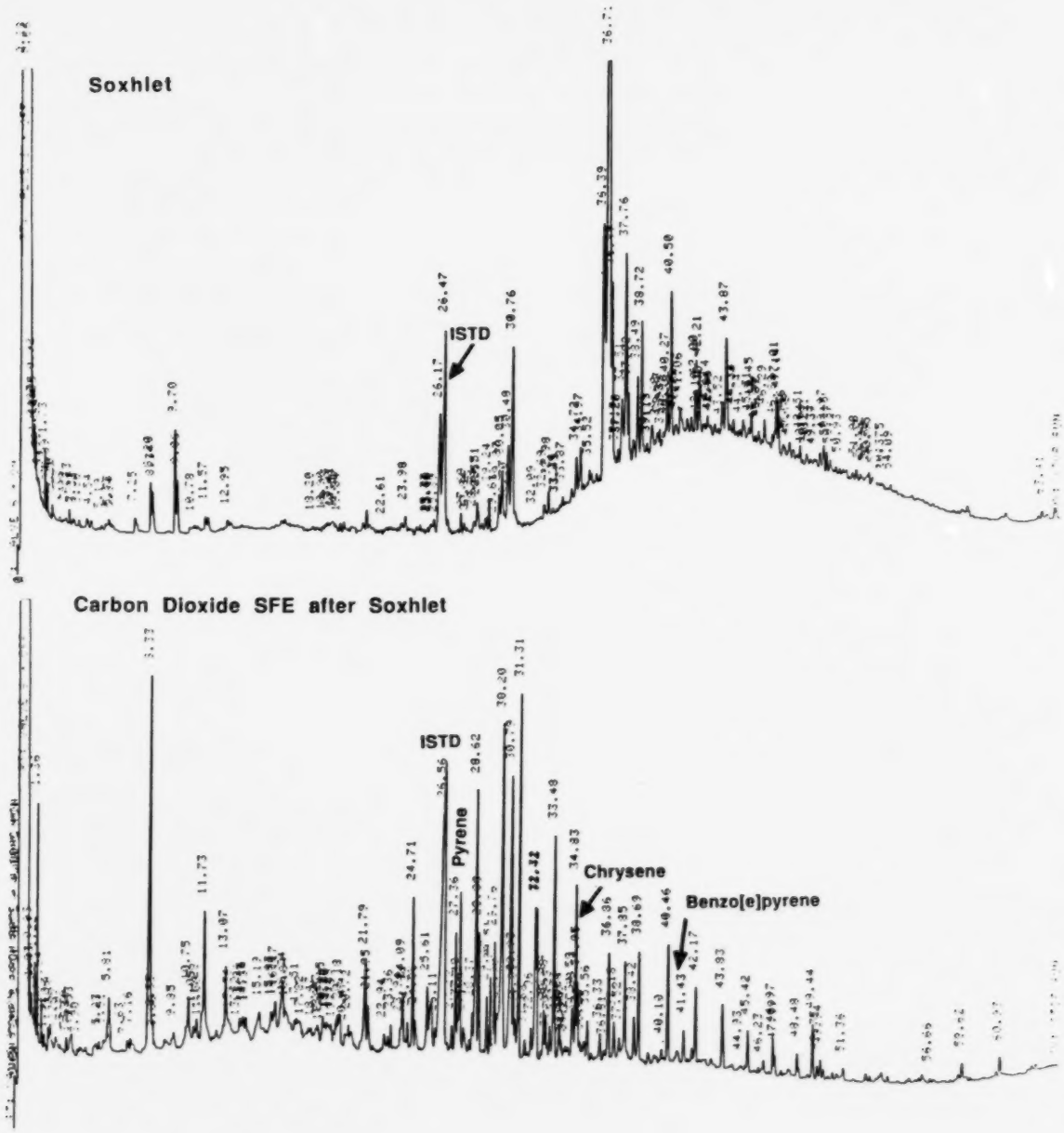

Figure 7. Capillary gas chromatograms of the Soxhlet extract and the carbon dioxide SFE after Soxhlet extract of NBS Urban Dust.

TABLE 8. EXTRACTION COMPARISON OF URBAN DUST USING SOXHLET AND ISOBUTANE SFE

	Recovery (μg/g, ± 10 %)				
Compound[a]	Sox.[b]	SFE[c] After Sox.	SFE[c]	Sox.[b] After SFE	NBS
Acenaphthylene	<0.01	1.1	<0.01	0.4	--
Phenanathrene	2.8	2.1	2.7	<0.01	4.5
Pyrene	1.6	0.2	2.2	<0.01	6.2-7.2
Chrysene	5.3	0.2	3.8	<0.01	3.5-3.7
Benzo[ghi]perylene	2.7	0.5	1.5	<0.01	4.5 (Cert.)

[a]Compounds identified on the basis of GC retention time only.

[b]Methylene Chloride for 16 hrs.

[c]Isobutane at 150°C and 185 bar with ~400 mL of liquid.

proved to recover more PAH material. It should be re-emphasized that the SFE extractions were done in a thirty to forty-five minute time frame with limited volumes of extracting fluid. Larger extracting fluid volumes would have undoubedly increased the overall amount of material that could be extracted, perhaps to the point where no additional extractable (by either Soxhlet or SFE methods) PAH could be recovered.

ON-LINE SUPERCRITICAL FLUID EXTRACTION-GAS CHROMATOGRAPHY

Typically, sample preparation and fractionation requires several procedures which may be more time consuming than the actual analysis. The automated on-line combination of SFE with high resolution capillary column gas chromatography provides a new method of overcoming this undesirable situation. Capillary gas chromatography (GC) was utilized for the analysis method in this study since the types of components of interest were amenable to this technique. However, other chromatographic methods, including capillary supercritical fluid chromatography (SFC), would also be compatible with this concept. The use of SFC would allow application to a wider range of compounds than is possible with GC including thermally labile and less volatile

(polar and/or high molecular weight) species (19,20). Gas chromatography, however, provides simpler operation and higher efficiencies than feasible with SFC or HPLC and is amenable to the vast majority of compounds extractable with nonpolar fluids such as carbon dioxide. Several modes of operation are possible utilizing the on-line SFE-GC approach including quantitative extraction and analysis, selective extraction or fractionation, and periodic sampling (and analysis) of the extraction effluent at various pressures for qualitative characterization of a sample.

The selective solvating power of a supercritical fluid can be used to fractionate a sample during the extraction process. An example of on-line sample fractionation and analysis is shown in Figure 8. In this example, the PAC material adsorbed on glass beads was extracted for one minute at three progressively higher pressures with the effluent from each extraction being analyzed by temperature programmed capillary GC prior to the next extraction. During each GC analysis, the extraction process was continued (~ 75 minutes) with the effluent being vented to the collection reservoir. In this way, essentially all of the material which was soluble at each pressure was extracted from the matrix prior to the next higher extraction pressure. After the GC analysis of a specific extract was completed, the fluid pressure was raised to a new setpoint and after a five minute equilibration period the effluent was again directed to the chromatographic column. Carbon dioxide, which has a critical temperature of approximately 31°C and a critical pressure of 73 bar, was used as the extraction fluid. The extractions were all conducted at 50°C and at pressures of 80, 125, and 200 bar which corresponded to densities (proportional to the solvating power) of 0.23, 0.62, and 0.78 g/mL, respectively (21,22). The extraction effluent was collected and concentrated on-column at 30°C which proved adequate to focus the solute injection band. Collection at higher temperatures or without the retention gap resulted in broadened peaks and lower resolution separations. After an extraction step was completed the oven temperature was maintained at 30°C for two minutes before the GC analysis was accomplished by temperature programming at 4°C/min to a final temperature of 265°C.

Examination of the chromatograms in Figure 8 indicates that high resolution separations of three essentially unique fractions of the material were obtained. As expected, progressively higher molecular weight material was extracted with the higher density extraction fluid. Since this material was essentially all polycyclic aromatic hydrocarbons, a separation based on molecular weight would be expected rather than a distribution characteristic of a class-selective fractionation. The peaks labeled "A" and "B" refer to the same respective components in each fraction and were arbitrarily chosen to serve as reference points to facilitate comparison. Although some overlap of components occurred in the different fractions, this example clearly demonstrates the potential of this method for reasonably efficient on-line fractionations and analyses. The components in the 80 bar fraction consisted primarily of two-ring, alkylated

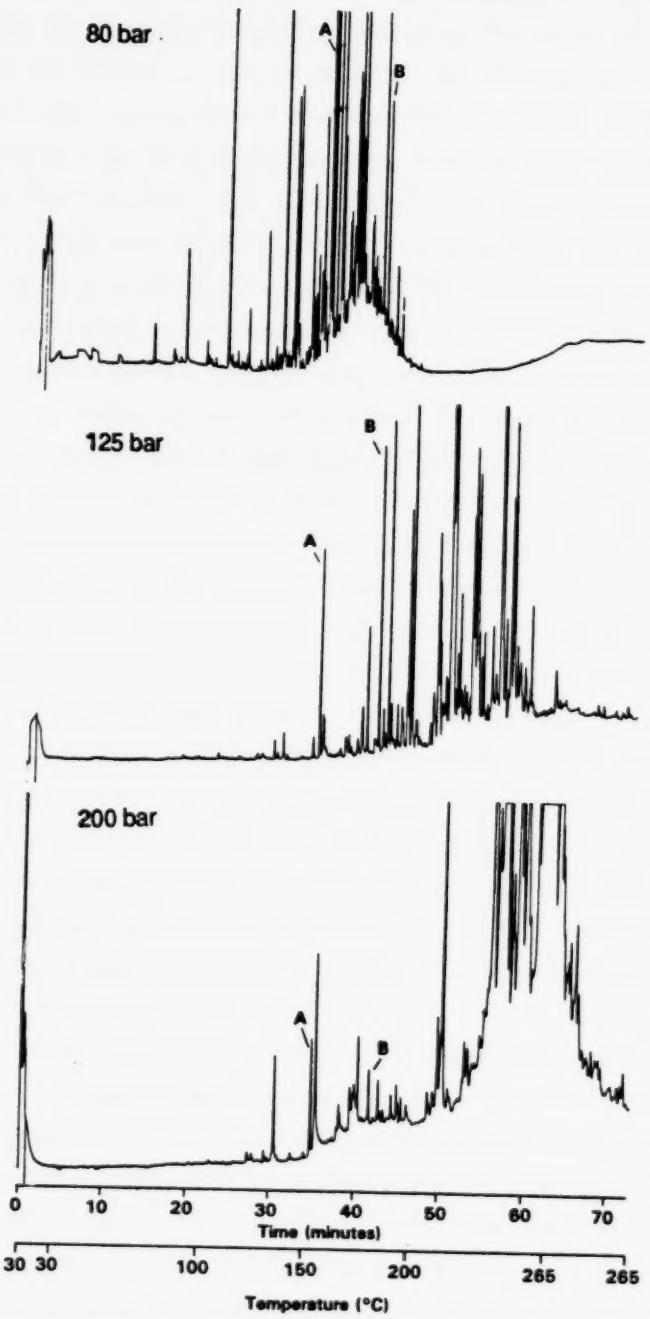

Figure 8. Capillary gas chromatograms of PAC fractions obtained from supercritical carbon dioxide extraction of a complex matrix at various pressures.

two-ring, three-ring, and some lower alkylated three-ring compounds. The 125 bar fraction consisted predominately of alkylated three-ring, four-ring, and some alkylated four-ring structures and the 200 bar fraction consisted primarily of alkylated four-ring and larger structures. More distinct fractions could have been obtained with longer (or faster fluid flow) extractions at each pressure. The rate of extraction is limited by the solubility of the compounds in the fluid and the volume of fluid used in the extraction. Thus, samples with higher component concentrations require longer extraction times (or larger fluid volumes) or a stronger solvating fluid for complete extraction. Very slow fluid flows were purposely used in these extractions, but faster flow rates could have been used to obtain more rapid extraction rates.

The selective extraction capability that can be achieved as a function of fluid pressure is further illustrated with the analyses shown in Figure 9 of PAH from the glass beads. In this example, each chromatogram was obtained from the progressively higher pressure extraction of the glass beads that were spiked with 25 ppm of each PAH. Carbon dioxide at 50°C was used as the extraction fluid. As in the previous example, the extraction effluent was directed into the GC column for one minute and then to the collection reservoir at the specified pressure for the duration of the GC analysis. The oven temperature was held at 30°C during the extraction and for an additional two minutes before the temperature programmed GC analysis was initiated. The temperature was programmed at 4°C/min to 280°C. When the extraction was conducted at 75 bar (density = 0.20 g/mL), essentially only the naphthalene and the fluorene were extracted. (A smaller than expected naphthalene peak was obtained due to volatility losses that presumably occurred during sample preparation.) At the next extraction pressure of 90 bar (density = 0.30 g/mL) essentially all of the naphthalene was exhausted and phenanthrene was the major component along with a significant quantity of pyrene. At 110 bar (density = 0.52 g/mL), pyrene was the only major component that was extracted, although low levels of the previous components were also still present. At 150 bar (density = 0.71 g/mL), chrysene and benzo[e]pyrene were the major components extracted. The tailing peaks adjoining the chrysene and benzo[e]pyrene peaks are contaminants that were extracted from rubber O-ring material that inadvertently entered the extraction cell. These extractions obtained under arbitrarily chosen conditions illustrate that very selective extraction of specific components could be obtained by optimizing the extraction conditions. Extraction at low density could be used to remove unwanted components and then extraction at the minimum density necessary to obtain the desired analytes would produce a simplified fraction. Trace levels of the desired analytes could also be concentrated in this specific fraction since the bulk of the material would not be subjected to chromatographic analysis. In addition to pressure to control the density, temperature could also

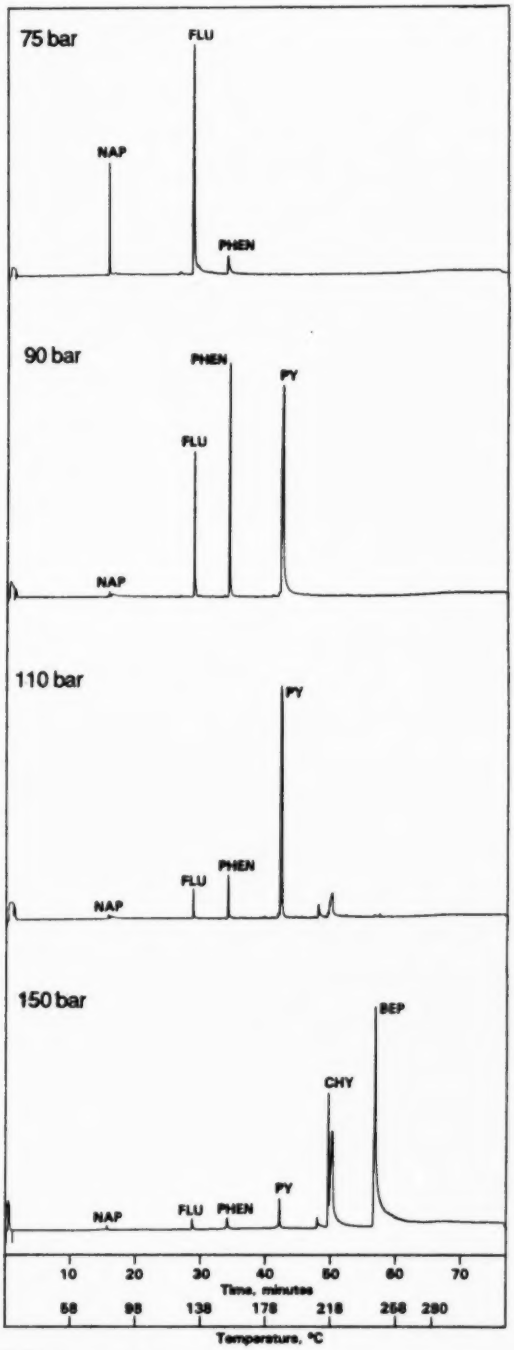

Figure 9. Capillary gas chromatograms obtained from the supercritical carbon dioxide extraction of a PAH standard adsorbed on glass beads at various pressures. NAP=naphthalene, FLU=fluorene, PHEN=phenanthrene, PY=pyrene, CHY= chrysene, BEP=benzo[e]pyrene.

be manipulated to take advantage of both density and volatility effects. The use of other supercritical fluids with a range of solvating properties could also be used to obtain the specific selectivity desired. Finally, different polarity chromatographic columns could be used to further enhance the selectivity of the separation in a similar manner as is used in two-dimensional chromatography.

Exhaustive extraction of a matrix at higher pressures where all of the components are soluble provides the potential for quantitative analysis. This is illustrated by the example shown in Figure 10 in which glass beads spiked with a low level (0.5 ppm) of the PAH compounds were subjected to exhaustive extraction with the entire effluent being deposited on-column for analysis. The extraction was conducted for two minutes with carbon dioxide at 50°C and 250 bar (density = 0.83 g/mL). After extraction, the fluid flow was stopped rather than being switched to the collection port. Due to the small sample cell size (15 µl), the low analyte levels (~4 ng), and a relatively higher fluid flow rate (x10), an exhaustive extraction was obtained very rapidly. Longer extraction times would have been necessary for larger samples. The oven temperature was held at 30°C during extraction and then the GC analysis was accomplished by temperature programming at 4°C/min to a final temperature of 280°C. Except for the naphthalene, all of the components in the mixture were found at similar concentration levels. Significant losses of naphthalene probably occurred through volatility during sample preparation. Since the detector response was not rigorously calibrated, quantification of the sample components could not be performed. However, since no detectable levels of PAH were recovered when a second extraction was applied, complete removal of the PAH components likely occurred with the first extraction.

The application of on-line extraction-gas chromatography for sample preparation and analysis of air particulate matter is illustrated in Figure 11. The chromatograms shown in this example were obtained from the progressively higher pressure supercritical fluid extraction steps of NBS Urban Dust. Similar extraction and chromatography parameters as described in Figure 9 were utilized. At an extraction pressure of 80 bar (density = 0.23 g/mL) negligible amounts of the compounds were extracted from the dust particles. It is interesting to note that extraction of the glass beads spiked with the PAC material at similar solvating power conditions removed a significant amount of material (see Figure 8). This probably illustrates the stronger matrix effect on extraction efficiency of the dust particles. At 120 atmospheres (density = 0.60 g/mL), where much higher solvating conditions were obtained, a complex fraction of material was extracted. Going to 160 atmospheres where somewhat higher solvating power was obtained (density = 0.72 g/mL), a significantly greater amount of material was further extracted which illustrates the overall complexity of the matrix. Extraction at 200 atmospheres (density = 0.78)

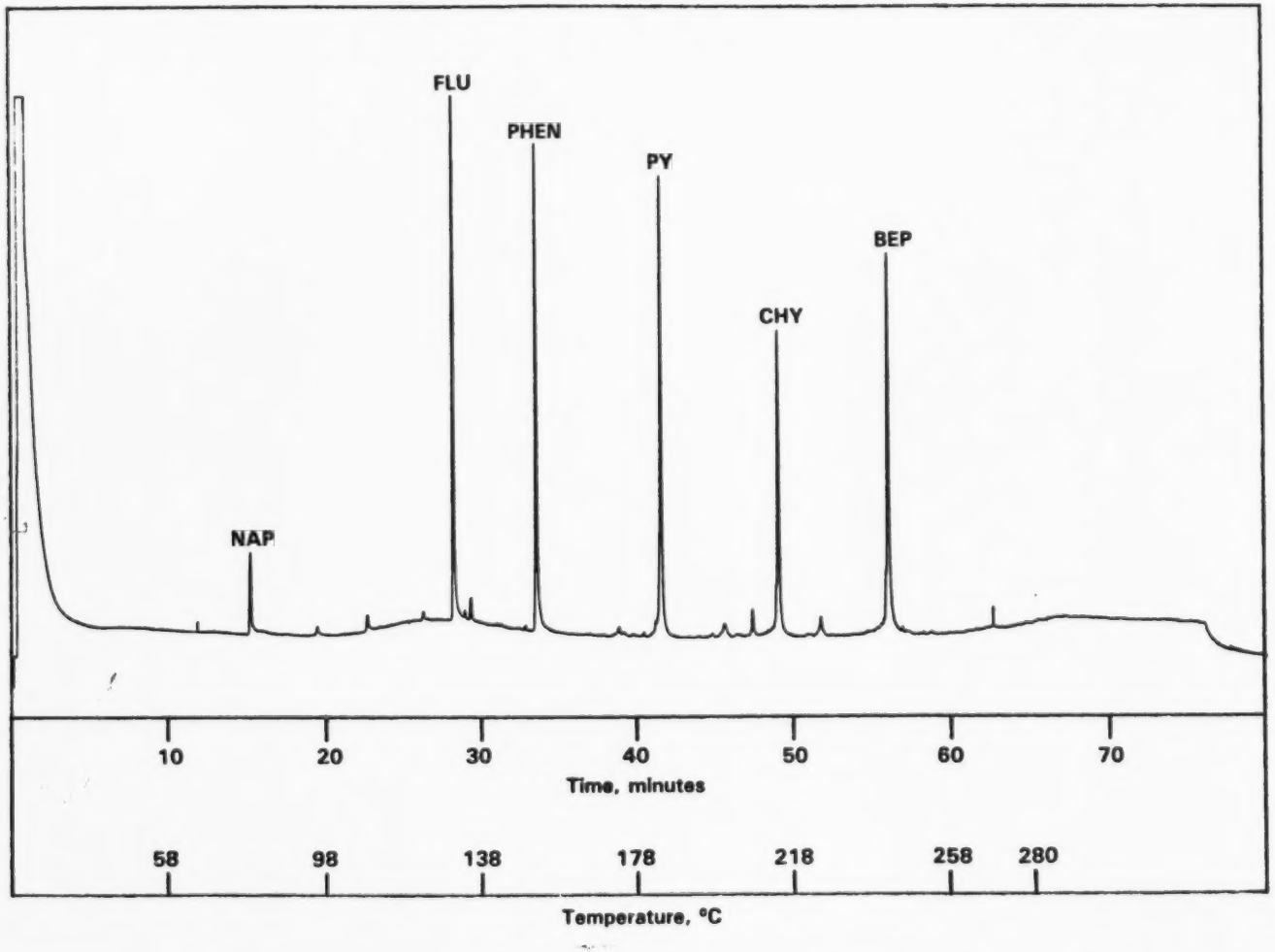

Figure 10. Capillary gas chromatogram obtained from the supercritical carbon dioxide extraction of the PAH standard at 250 bar. Peak identifications the same as in Figure 9.

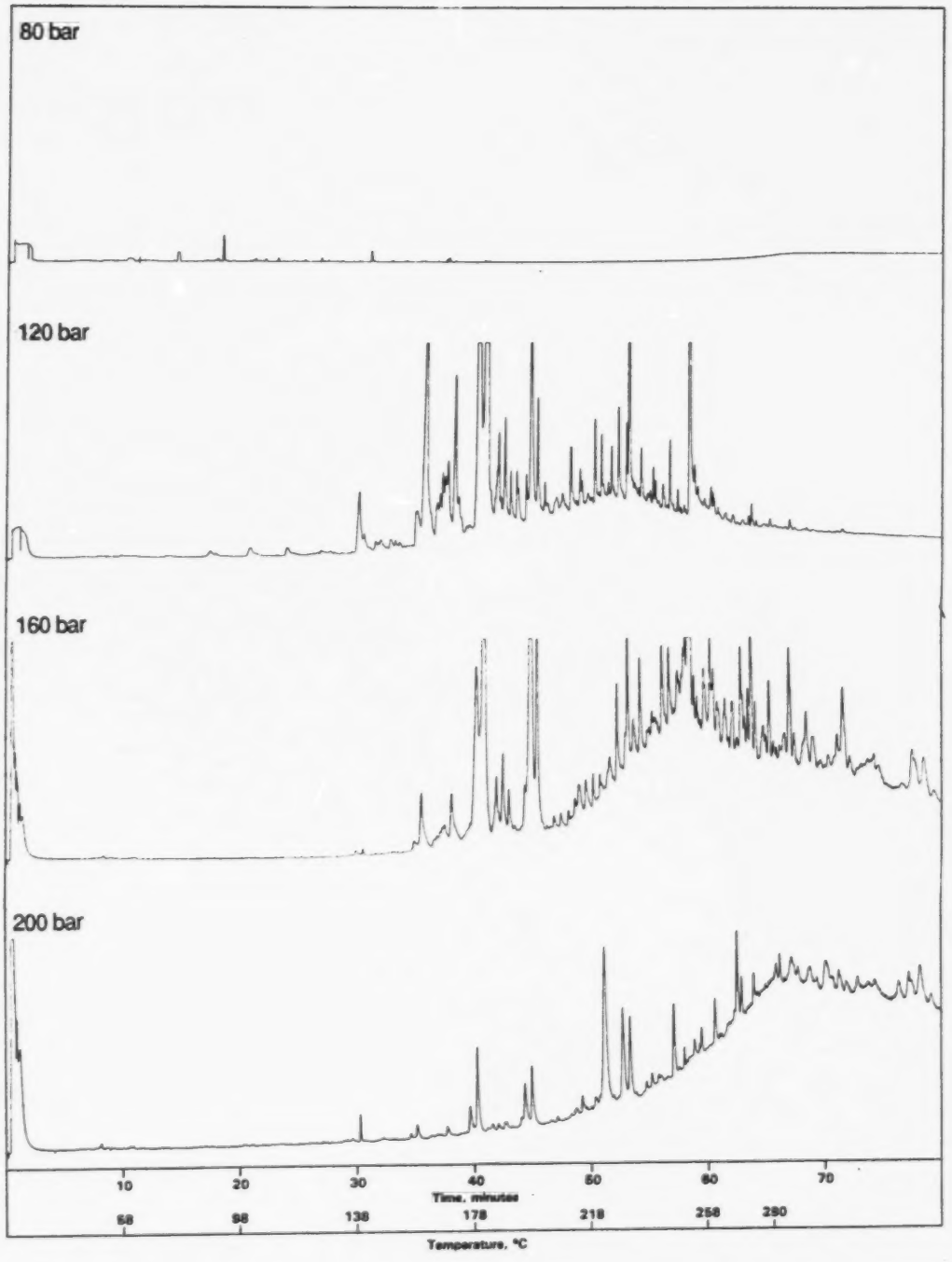

Figure 11. Capillary gas chromatograms of various fractions obtained from the supercritical carbon dioxide extraction of Urban Dust particles at various pressures.

removed additional higher molecular weight material that is particularly evident in the unresolved "hump" near the end of the chromatogram. However, the lower molecular weight material was nearly exhausted and the overall complexity of the resolved components in the chromatogram was significantly reduced. Due to the complexity of the sample matrix and the method of extraction used, exhaustive extractions were not obtained for the the individual analyses at each pressure. A smaller sample size, increased extraction times between GC analyses, or increased flow through the collection reservoir restrictor would have allowed more exhaustive extraction. However, in many instances, exhaustive extraction is not necessary for qualitative sample fractionation.

Operation of the SFE-GC methodology in a truly quantitative mode in which the detector had been calibrated is illustrated with the example shown in Figure 12. XAD-2 resin that had been spiked with approximately 7.5 ng of each compound from the PAH standard mixture was extracted at 325 bar and 50°C for seven minutes. The entire extraction effluent was directed to the chromatographic column and a sufficiently large volume of extraction fluid was utilized to obtain an exhaustive extraction (the gas flow rate was approximately 250 mL/min). The oven was held at 0°C during the extraction and then programmed at 15°C/min to 50°C and then at 6°C/min to 265 °C to effect the chromatographic analysis. The detector response was calibrated for each component immediately prior to the extraction. The spike levels and recovered quantities of each PAH component are listed in Table 9. Within the experimental error limit (~15%), quantitative extraction and recovery of all the compounds, except for the benzo[e]pyrene, was obtained. Benzo[e]pyrene has lower solubility in carbon dioxide and was not completely extracted with the fluid volume utilized. However, the remaining benzo[e]pyrene was recovered with a second extraction. The extraneous peaks in the chromatogram can be attributed to impurities concentrated from the carbon dioxide or to impurities extracted from the XAD-2 resin.

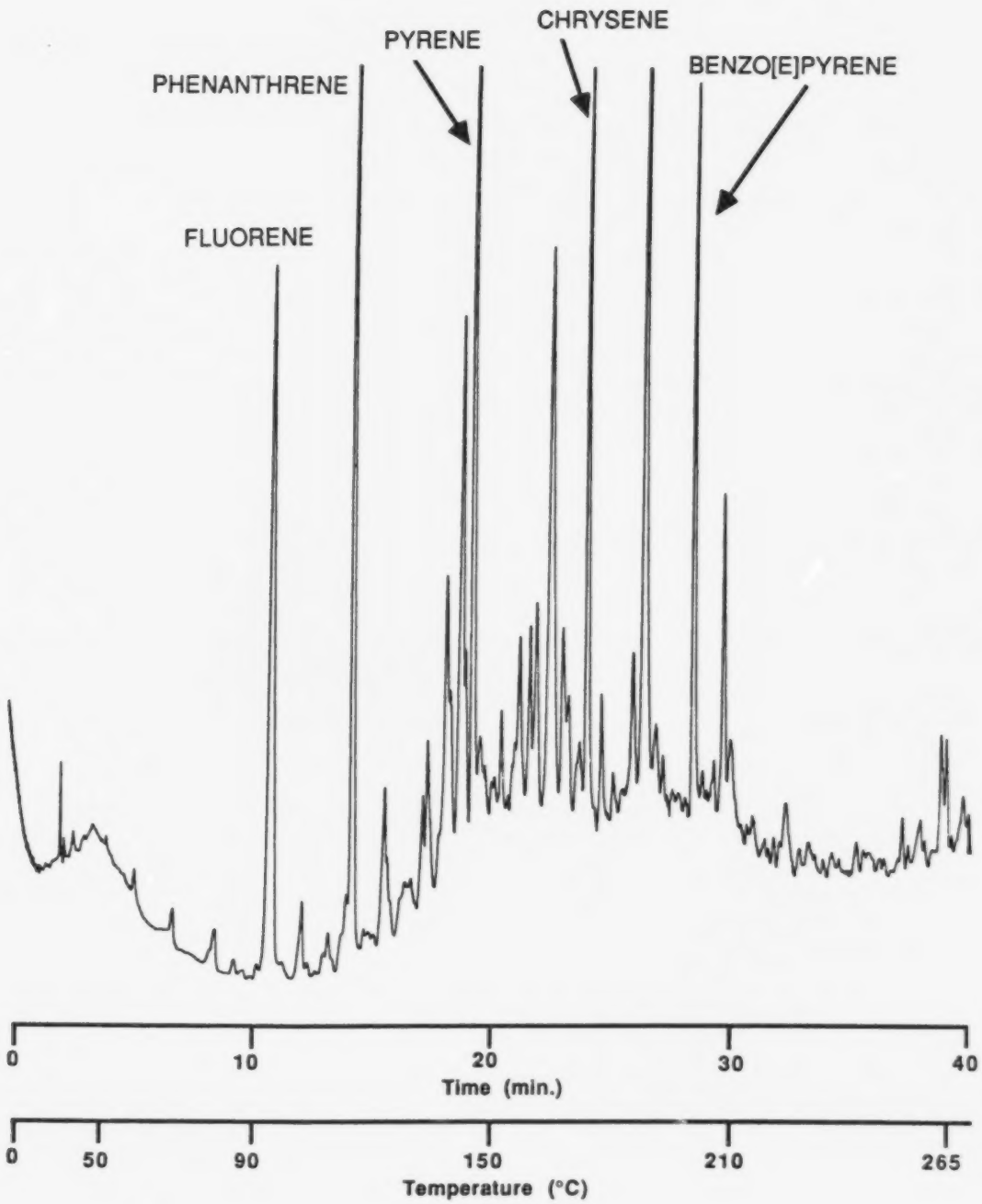

Figure 12. Capillary gas chromatogram obtained from the supercritical carbon dioxide extraction of XAD-2 resin.

TABLE 9. RECOVERY OF PAH STANDARD FROM XAD-2 RESIN

Compound	Spike Level (ng)	Recovered Amount (ng)	% Recovery
Fluorene	7.6	7.6	100
Phenanthrene	7.4	7.1	96
Pyrene	7.5	6.6	88
Chrysene	7.3	6.4	88
Benzo[e]pyrene	7.4	4.9	66

DIRECT SUPERCRITICAL FLUID EXTRACTION-MASS SPECTROMETRY

To better understand the extraction process of the model compounds from the various adsorbents with the different fluid systems, the extraction kinetics were monitored by direct supercritical fluid extraction-mass spectrometry. This process also provided information that would be valuable in optimizing selective extractions. In general, the extraction profiles (relative intensity of the major ion from each component versus time) showed increased solubility for each compound as the pressure (and solvent power) was increased, and then a decline in intensity with time as the material was exhausted. Thus, this approach provides a basis to study the kinetics of the extraction process for individual compounds even in complex matrices. The specific adsorbents and fluid systems are described below.

XAD-2 Resin

The extraction profiles of the model compounds obtained from carbon dioxide extraction of XAD-2 resin are shown in Figure 13. An extraction temperature of 50°C was utilized and the pressure was ramped from 114 to 370 bar at 20 bar/min. Consequently, the upper pressure and maximum solvating power conditions were reached at approximately thirteen minutes. Isobutane was used for the chemical ionization reagent gas and intense protonated molecular ions, $(M+1)^+$, were achieved for each of the model compounds. The expected rearrangement ion, $(M+1-30)^+$, was obtained for 1-nitropyrene. The normalization factors for the single ion plots in each extraction were kept constant so a comparison between signal intensities could be made.

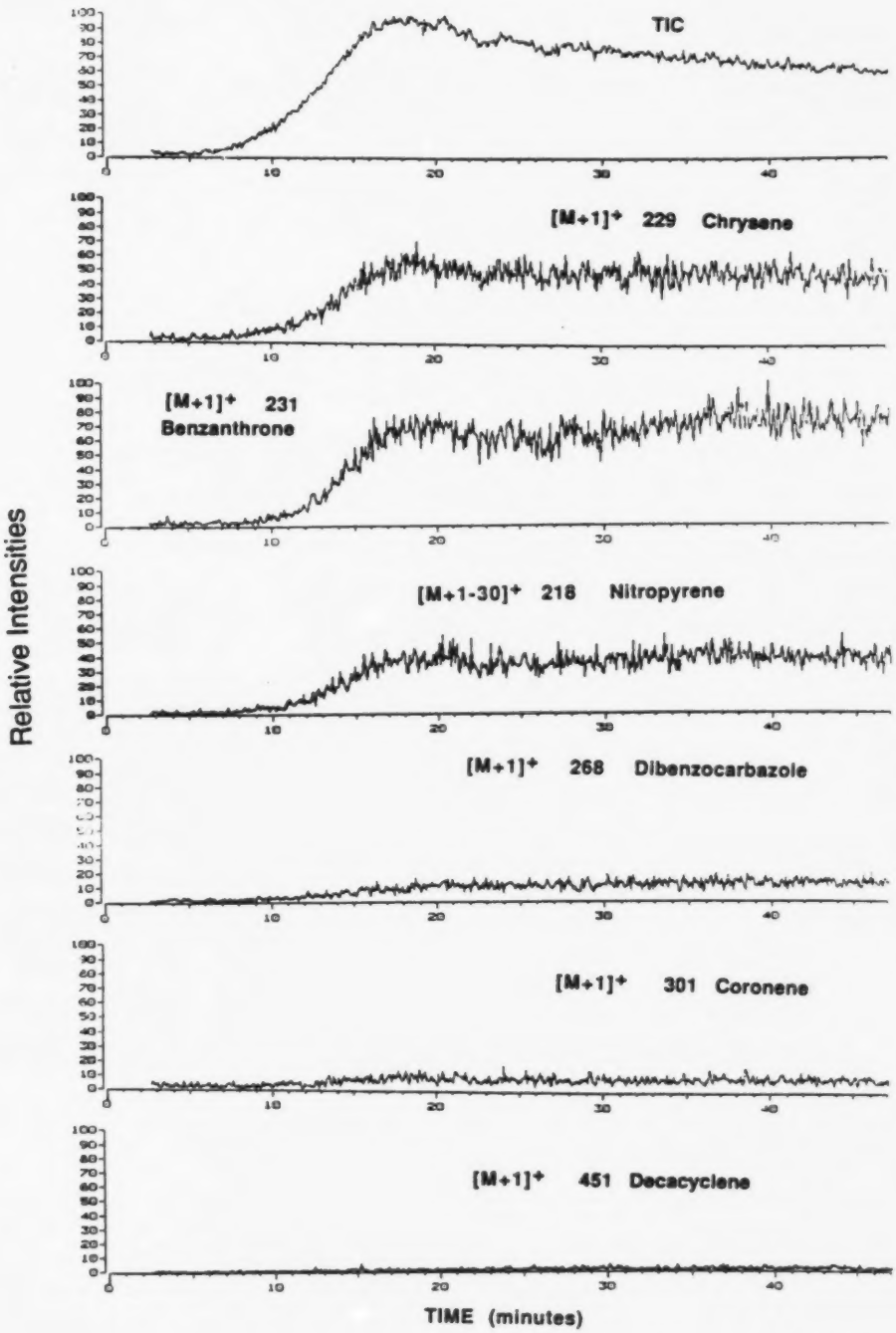

Figure 13. Extraction profiles of the model compounds obtained by SFE-MS with carbon dioxide from XAD-2 resin.

Although the instrument response factors vary for the individual compounds, comparison of the signal intensities provides a direct measure of the relative solubilities (and extractabilities) of the compounds. As the solvating power of the extracting fluid was increased, the signal intensities for each of the compounds also increased with the steepest slopes observed for the more soluble compounds. Interestingly, the signal intensities continued to increase for approximately five minutes after the maximum pressure was reached which can probably be accounted for by the time delay associated with the flow from the extraction cell to the mass spectrometer and from equilibration of the fluid with the matrix due to mass transport considerations. Since the maximum signal intensity was observed at approximately the same time for all the compounds, it is not likely that the solubility limit was exceeded for the more soluble components. Chrysene and benzanthrone were most readily extracted and had the highest solubility in supercritical carbon dioxide. As expected, the higher molecular weight decacyclene and coronene displayed much lower solubility. Exhaustion of the compounds occurred slowly with only minor decreases in the signal intensity observed with time. A more pronounced decrease can be observed in the total ion current (TIC) plot. Greater decreases would have resulted with a longer extraction time or higher flow rate.

Similar plots for the extraction of the model compounds from XAD-2 resin using isobutane as the fluid are shown in Figure 14. Since isobutane has a higher critical temperature, this extraction was conducted at 150°C or a reduced temperature of 1.03 which is similar to the reduced temperture of 1.06 utilized for the carbon dioxide extraction. The pressure was ramped from 114 to 195 bar at 10 bar/min. Thus, the maximum solvating power conditions were achieved at approximately eight minutes. Again, the maximum signal intensities were not observed until several minutes after the maximum pressure had been reached. The signal maximum for dibenzocarbazole was shifted to an even later time. Overall better extractability of the model compounds was achieved with isobutane than with the carbon dioxide, as evidenced by the higher signal intensities for the more polar and higher molecular weight compounds. Exhaustion of the compounds from the matrix could also be clearly observed as the signal intensities of the extraction profiles decreased to near zero levels in approximately thirty minutes. Better extractability can be accounted for by higher solubility of the compounds in isobutane and by the higher extraction temperature, which would weaken the adsorption interactions of the matrix. The large envelope beginning at approximately three minutes and falling sharply at twelve minutes in the coronene extraction profile suggests that a contaminant (with the same molecular weight) may have been present that was more easily extracted was also present. The lower intensity curve visible after twelve minutes is more typical of the expected profile. A very low signal intensity was observed for the decacyclene which may be a result of a low relative

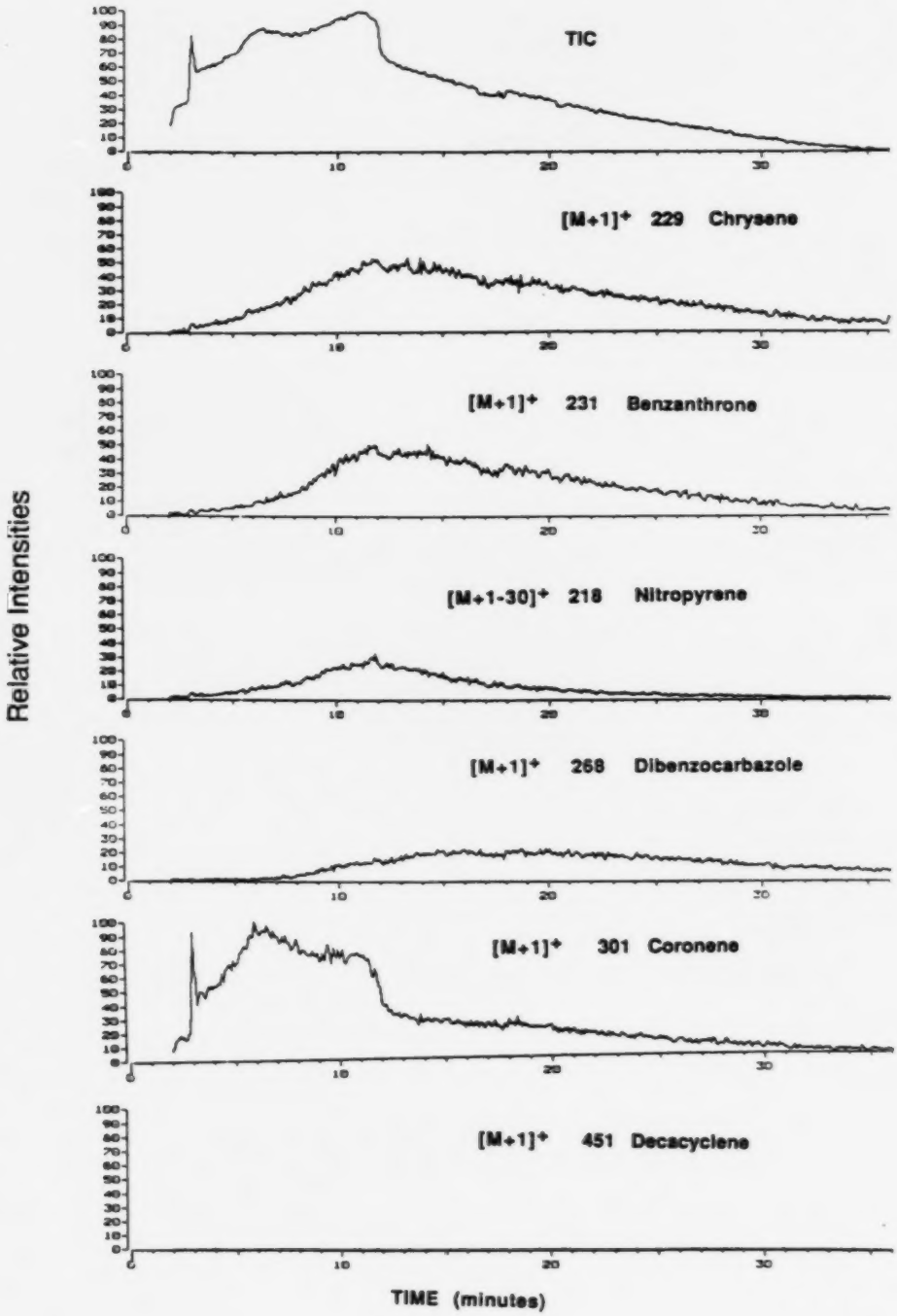

Figure 14. Extraction profiles of the model compounds obtained by SFE-MS with isobutane from XAD-2 resin.

abundance of $(M+1)^+$ ions being formed under the specific chemical ionization conditions rather than a very low solubility and extractability in isobutane.

The extraction profiles of the model compounds from XAD-2 resin obtained from methanol-modified (18.2 mole %) carbon dioxide extraction are shown in Figure 15. Unfortunately, this extraction was done before the critical phase behavior of this mixture was established and a subcritical temperature of 105°C was utilized. The critical temperature of this mixture is approximately 120°C (16). Consequently, a two-phase mixture possibly enriched in liquid methanol was being used as the extraction medium which would be expected to behave differently from a supercritical fluid. One difference would be that an increase in pressure would not necessarily increase the solvating power of the mixture. The pressure was ramped from 135 to 400 bar at 20 bar/min so the maximum pressure was not reached until approximately thirteen minutes. However, the extraction profile maxima were reached several minutes earlier, which is consistent with the expected behavior of a two-phase mixture. The best extractabilities (highest extraction profile intensities) were obtained for benzanthrone and dibenzocarbazole, which are capable of hydrogen bonding interactions and would render them more soluble in a methanol enriched liquid phase. More distinct decay profiles were also obtained for these two compounds as they quickly achieved high solubility and were exhausted from the matrix. The neutral PAH compounds (chrysene, coronene, and decacyclene) exhibited much lower solubilities. It would be interesting to contrast these extraction profiles obtained with a two-phase mixture to those obtained with an actual binary supercritical fluid mixture.

PUF Matrix

The extraction profiles of the model compounds from the PUF matrix using carbon dioxide are shown in Figure 16. This extraction was conducted at 50°C and the pressure was ramped from 114 to 370 bar at 20 bar/min. Consequently, the maximum solvating power was reached at approximately thirteen minutes. A delay was observed before the maximum signal intensities were achieved due to mixing, mass transport, and the time required for the fluid to flow from the extraction cell to the mass spectrometer. The extraction profile shapes were similar for all of the compounds with distinct maxima being reached followed by a reduction in intensity as the material was exhausted. The steepest slopes on the rising edges of the profiles were obtained for the more soluble compounds and the shallowest slopes on the tailing edges of the profiles were observed for the compounds of lower solubility. The absolute signal intensities also corresponded to the expected solubility order of the compounds with the lower molecular weight and least polar compounds being most soluble and the higher molecular weight polar compounds the least soluble. It is interesting to contrast these extraction profiles obtained from the PUF matrix to those obtained from the XAD-2 resin shown in Figure 13. More defined curves with

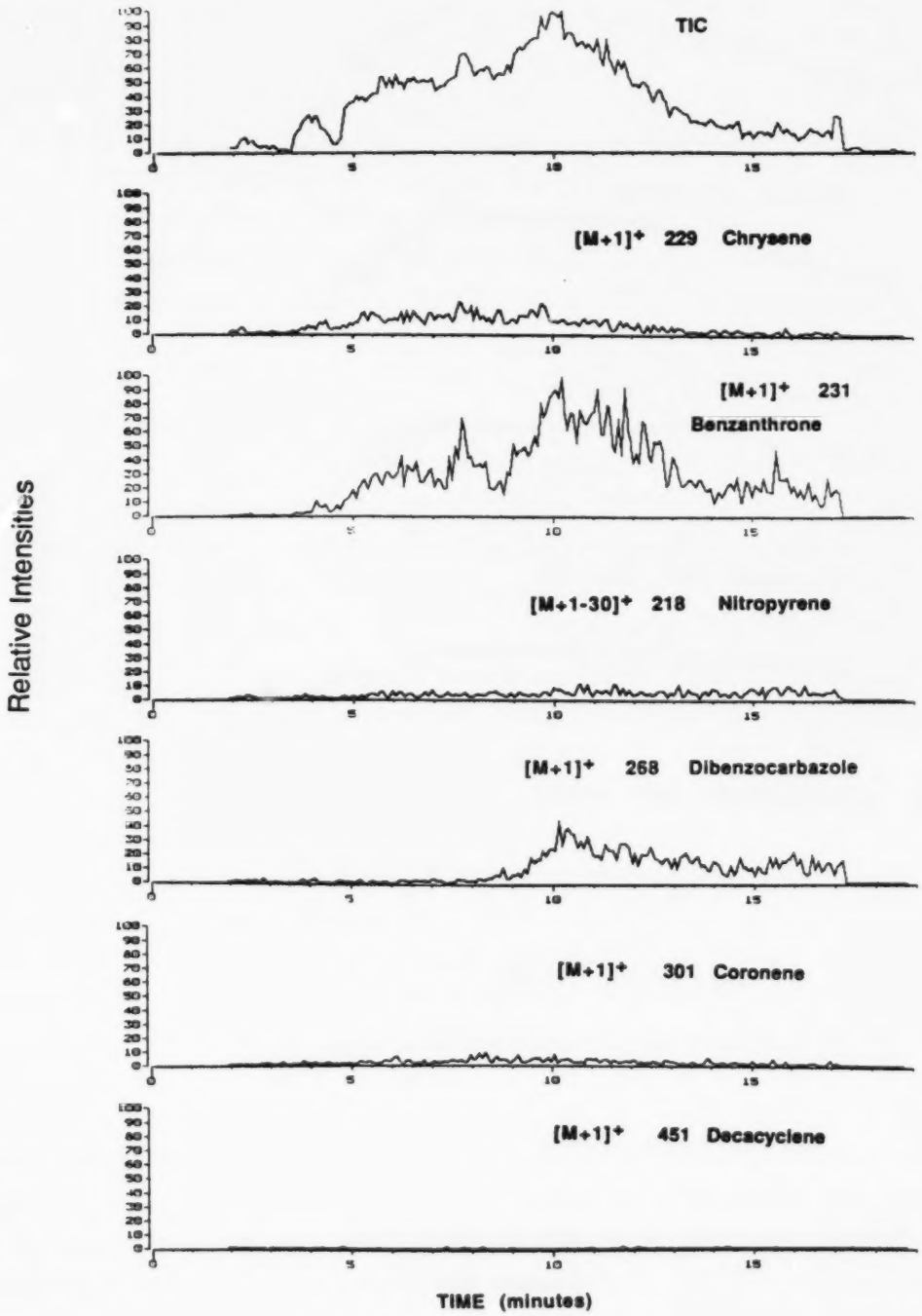

Figure 15. Extraction profiles of the model compounds obtained by SFE-MS with methanol modified carbon dioxide from XAD-2 resin.

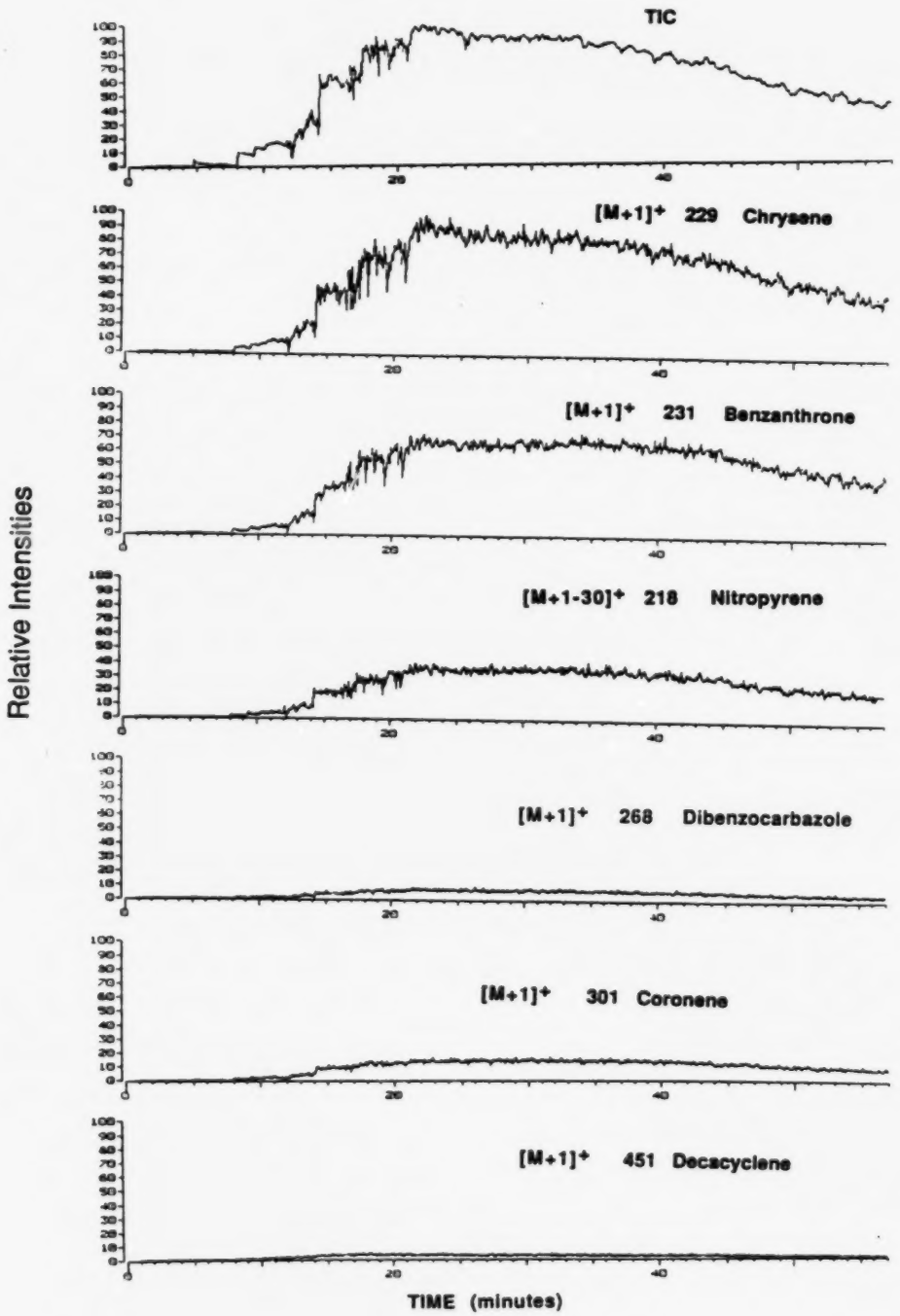

Figure 16. Extraction profiles of the model compounds obtained by SFE-MS with carbon dioxide from PUF.

distinct exhaustion behavior were obtained from the PUF matrix suggesting the compounds were more easily removed from the matrix. Thus, the intensities of the profiles would more nearly correspond to compound solubility rather than a combination of solubility and extractability.

The extraction profiles of the model compounds from the PUF matrix obtained with isobutane are shown in Figure 17. This extraction was conducted at 150°C and the pressure was ramped from 114 to 195 bar at 10 bar/min. Thus, the maximum solvating power conditions were achieved by eight minutes. Very steep extraction profiles for the compounds were obtained indicating high solubility in the fluid and easy extractability from the matrix. Again, the steepest rising slopes and highest intensities were obtained for the more soluble compounds. The tails of the profiles also fell rapidly suggesting that the material was being exhausted from the matrix. Curiously, a second rising profile of lower intensity was obtained at approximately twenty-seven minutes. This could possibly be accounted for by an inhomogeneously spiked sample with the time delay associated with the dead-volume of the extraction system or possible degradation of the PUF matrix. The rising slopes were steeper for these profiles than obtained for the profiles from the isobutane extraction of XAD-2 which again suggests (as expected) that the compounds were less tightly bound to the PUF matrix than the XAD-2 resin. These results are consistent with those obtained from the off-line extraction studies previously discussed. It must be noted that the absolute intensities of the profiles between different extractions can not be quantitatively compared since different normalization factors were applied.

The extraction profiles for the model compounds from the PUF matrix obtained with methanol-modified carbon dioxide are shown in Figure 18. This extraction was also unfortunately conducted at 105°C which is below the critical point of the mixture. The profiles are similar to those obtained from the XAD-2 resin with the highest intensities obtained for the polar compounds subject to hydrogen bonding and the lowest intensities for the neutral compounds. The rising slopes of the profiles are steeper, however, which further demonstrates that the compounds were easier to extract from the PUF matrix.

Spherocarb®

The spiked Spherocarb® matrix was also subjected to SFE-MS analysis. However, the compounds were so strongly adsorbed and were so inefficiently extracted that only weak signals were obtained and meaningful extraction profiles could not be constructed. This behavior was anticipated from the results of the off-line extraction studies previously described.

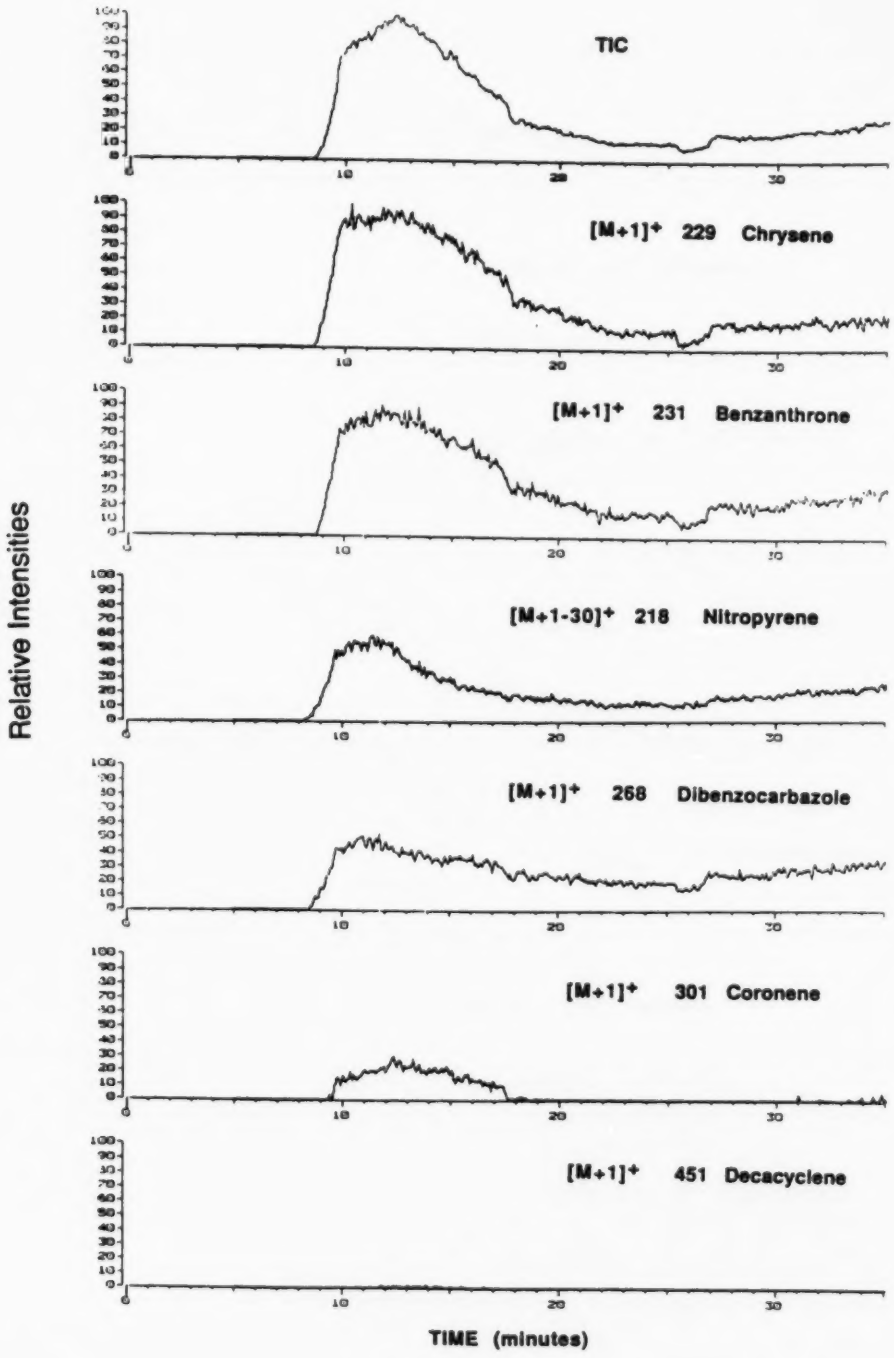

Figure 17. Extraction profiles of the model compounds obtained by SFE-MS with isobutane from PUF.

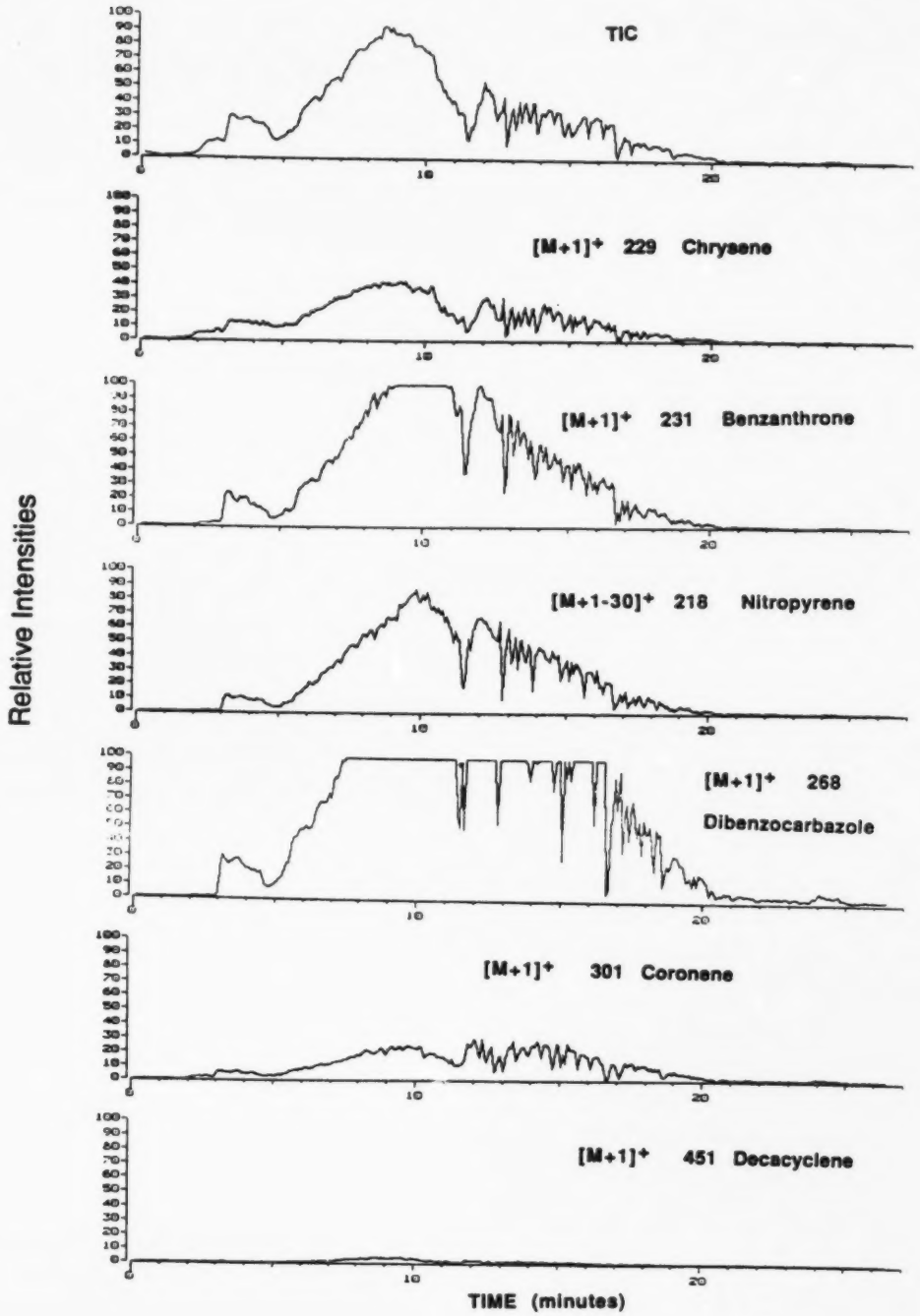

Figure 18. Extraction profiles of the model compounds obtained by SFE-MS with methanol modified carbon dioxide from PUF.

QUANTITATIVE FLUORESCENCE DETECTION SUPERCRITICAL FLUID CHROMATOGRAPHY

The evaluation of the quantitative capability of the fluorescence detection supercritical fluid chromatography involved three interrelated comparisons. They included an evaluation of a multi-level detector calibration of several compounds, the reproducibility of multiple determinations of specific mixtures, and a comparison of the quantitative values obtained from parallel determinations using this methodology and capillary gas chromatography. The quantitative performance of the split injection method that was used for sample introduction was also mirrored in all of these evaluations.

Detector Calibration

A typical chromatogram of the HMWPAC model compound mixture obtained using fluorescence detection supercritical fluid chromatography is shown in Figure 19. This separation was obtained using supercritical pentane at 210°C as the mobile phase and a short 5 m x 50 μm i.d. fused silica capillary column. A 24 ppm mixture of the model compounds was used for injection, but only approximately 250 pg of each component was actually injected into the column due to the split injection method. An excitation wavelength of 270 nm was used with collection of the entire emission greater than 270 nm. Very high sensitivities and relatively good separation of the higher molecular weight components were achieved in approximately fifteen minutes as the pressure of the mobile phase was increased. The benzanthrone and nitropyrene eluted very near the benzene solvent peak and were not used in the quantitative evaluations.

To evaluate the linearity of the detector response over the concentration range of interest, calibration plots for each model compound based on multiple analyses of four injection concentration levels ranging from 6 to 48 ppm were constructed. Calibration was accomplished using both peak heights and peak areas for the signal response. These calibration plots are shown in Figures 20 and 21, respectively. Relative standard deviations for replicate injections ranged from as little as 0.4 % for the 48 ppm chrysene height to as much as 12.1 % for the 6 ppm chrysene height. Much of the scatter can undoubtedly be attributed to injection discrimination and poor reproducibility. The slopes of the calibration plots for the various compounds were quite different (factor of five) reflecting the different fluorescence efficiencies and widely varying response factors typical of fluorescence detection. More significant differences would be expected for less similar compounds. It is also interesting that the relative slopes of the compounds were different for the area and height plots. The relevant statistical data describing the quality of the calibration for each model compound are listed in Table 10. Slightly better calibration was achieved with peak height as evidenced by the correlation coefficients (Table 10) and lower relative standard errors of the slopes for the linear regression equations.

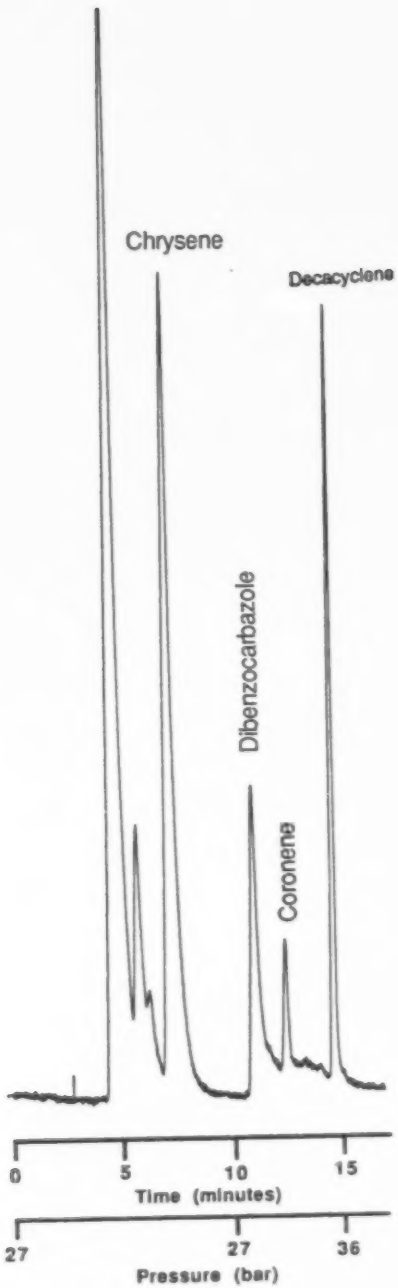

Figure 19. Typical calibration chromatogram of the model compound mixture obtained using fluorescence detection SFC.

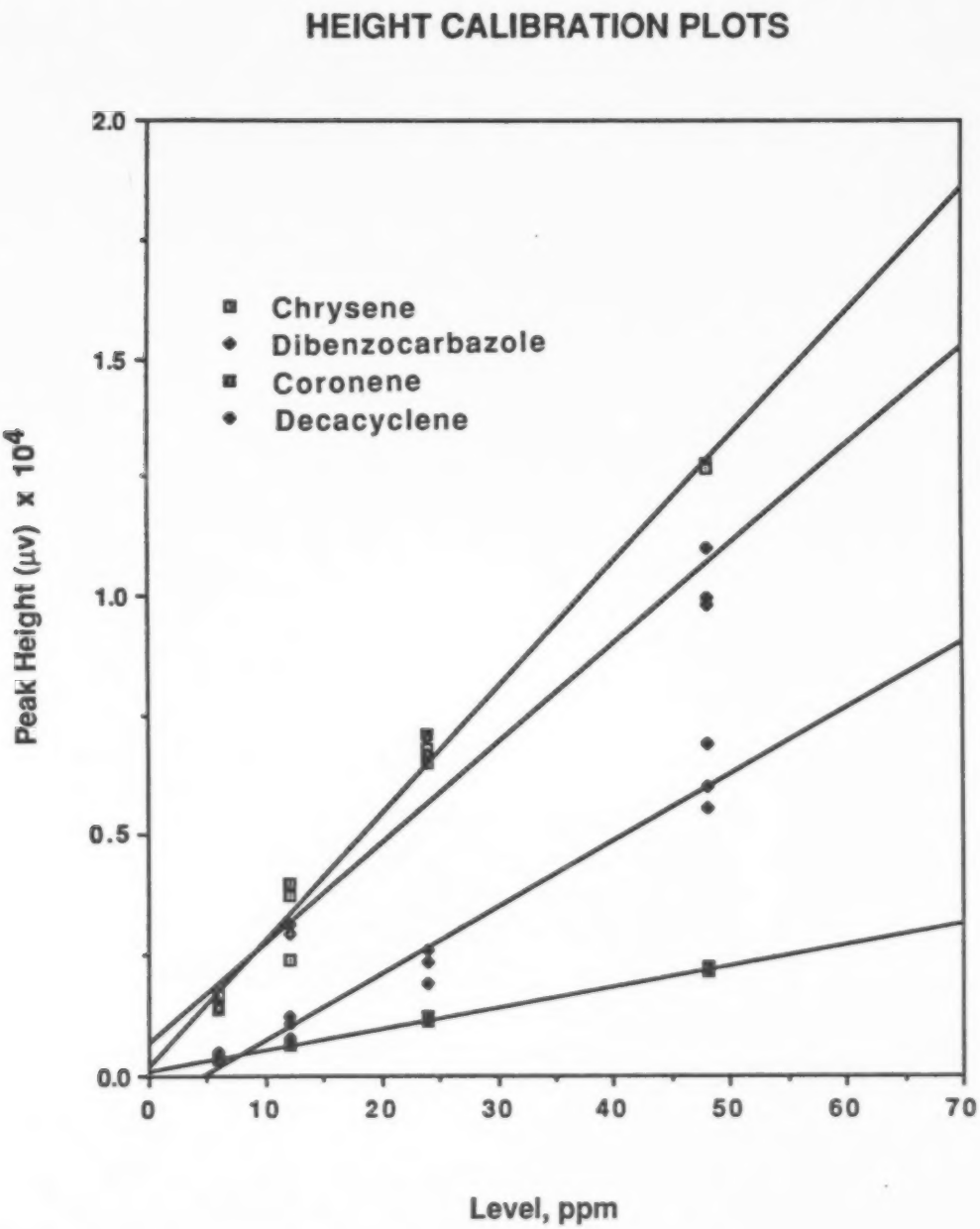

Figure 20. Fluorescence detection SFC calibration plots of the model compounds based on peak height.

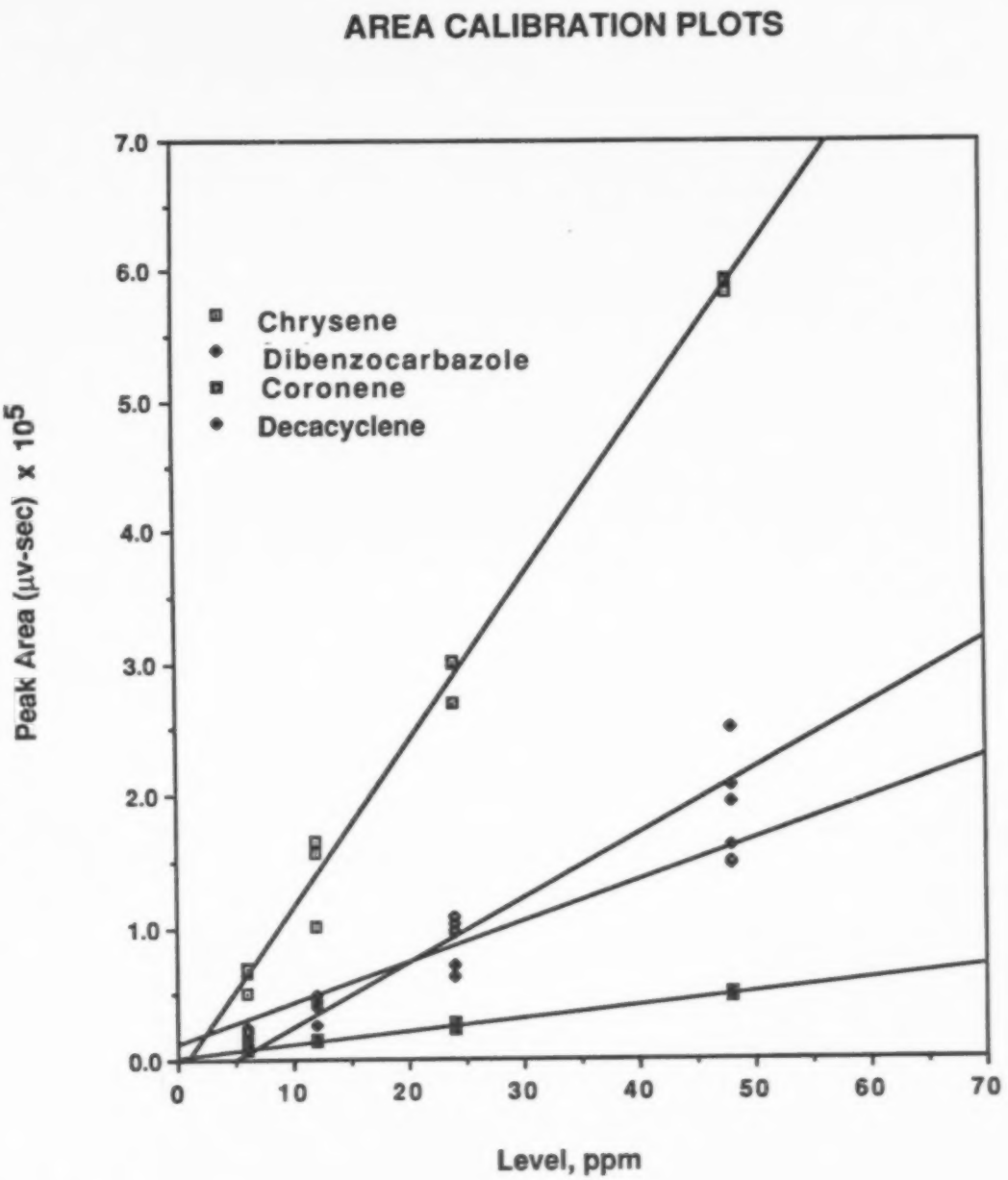

Figure 21. Fluorescence detection SFC calibration plots of the model compounds based on peak area.

TABLE 10. STATISTICS DATA FOR CALIBRATION PLOTS

Compound	Height Calibration		Area Calibration	
	Correlation Coefficient	Relative Standard Error	Correlation Coefficient	Relative Standard Error
Chrysene	0.995	3.1 %	0.997	2.7 %
Dibenzocarbazole	0.984	5.7 %	0.976	7.1 %
Coronene	0.998	1.9 %	0.994	3.5 %
Decacyclene	0.978	6.7 %	0.978	6.8 %

The stability of the detector response over a several day period was evaluated by analyzing the 24 ppm standard using the peak height calibration parameters. The detector was left operating from the time the calibration was performed until after the various analyses were completed. The data from these analyses are listed in Table 11. The relative errors from the expected values over the three days ranged up to 15 %. Although smaller errors would have been desirable, these measurements also reflect potential errors associated with the injection.

TABLE 11. DETECTOR RESPONSE STABILITY

Compound	Calculated Concentration Level (ppm)		
	Day 1	Day 2	Day 3
Chrysene	25.5	25.4	25.5
Dibenzocarbazole	28.7	26.1	27.4
Coronene	23.2	27.4	27.3
Decacyclene	23.1	27.2	26.0

Multiple Determinations

Actual sample extracts containing the model compounds were subjected to multiple analyses to evaluate the precision of the determined concentration levels. The same calibration parameters based on peak height as discussed previously were used for these determinations. The calculated concentrations of the model compounds from selected extracts are listed in Table 12. The relative standard deviation between replicate analyses ranged from less than 1.5 % to over 170 %. Generally, the replicate analyses that exhibited large variances included an analysis with drastically different values. These analyses would typically show low concentrations of only the early eluting components indicating that an improper injection was made. For actual determinations, these values could legitimately be eliminated, but for this evaluation they were included to present a worst case possibility. By eliminating the obviously outlying values, relative standard deviations of less than 15 % were generally obtained. Although this level of uncertainty and the potential errors associated with the calibration preclude high precision determinations, marginally acceptable quantitative analyses are currently feasible pending the development of improved methodologies. The injection method appears to account for the greatest source of potential error and deviation with this SFC method.

TABLE 12. PRECISION OF MULTIPLE SFC DETERMINATIONS

	Concentration (ppm)			
Sample	Chrysene	Dibenzocarbazole	Coronene	Decacyclene
XAD/SFE	35.0 ± 2.3	30.2 ± 0.4	13.4 ± 0.3	trace
XAD/Sox*	2.7 ± 1.4	4.4 ± 0.1	2.7 ± 3.8	32.1 ± 5.0
XAD/SFE	29.6 ± 17.0	23.0 ± 12.2	8.7 ± 7.6	trace
XAD/Sox	<0.05	18.2 ± 4.7	28.2 ± 2.1	33.4 ± 16.2
XAD/SFE	19.0 ± 8.9	15.7 ± 8.5	16.8 ± 14.6	0.9 ± 0.8
XAD/Sox	2.3 ± 3.2	4.4 ± 2.1	10.3 ± 0.1	44.3 ± 1.2
PUF/SFE	21.8 ± 0.3	23.6 ± 0.8	10.9 ± 1.8	1.2 ± 1.7
PUF/Sox	7.6 ± 0.2	trace	13.3 ± 1.2	45.8 ± 2.7
PUF/SFE	17.3 ± 14.3	8.7 ± 7.8	6.2 ± 10.7	1.5 ± 0.1
PUF/Sox	trace	0.8 ± 1.1	1.5 ± 2.1	15.4 ± 0.3

*Soxhlet Extraction

Parallel Analyses

The accuracy of the model compound determinations were checked by comparing the values to those obtained from quantitative capillary gas chromatographic analyses. Chrysene, carbazole, and coronene are amenable to gas chromatographic analysis and their concentration levels were determined during the off-line supercritical fluid extraction studies described earlier in this report. The concentration levels of the model compounds from selected extracts obtained by both GC and SFC methods are compared in Table 13. The SFC determination was chosen from the multiple analyses that exhibited the closest agreement to the GC determination. In general, good agreement (0-20% differences) between the SFC and GC determinations was achieved suggesting that the SFC method could provide accurate results if a gross injection problem was not encountered. In most cases, the GC value fell within the standard deviation range of the replicate SFC determinations (data not listed).

TABLE 13. GC AND SFC QUANTITATION COMPARISON

	GC Determination (ppm)			SFC Determination (ppm)		
Sample	Chrysene	Dibenzo-carbazole	Coronene	Chrysene	Dibenzo-Carbazole	Coronene
XAD/SFE	39.2	34.6	20.0	36.6	30.5	13.6
XAD/Sox*	5.7	8.7	17.4	3.7	4.5	5.4
XAD/SFE	38.5	26.8	17.4	48.5	36.2	13.9
XAD/Sox	<0.05	15.1	39.6	<0.05	14.9	38.6
XAD/SFE	31.4	21.1	26.5	28.4	25.3	26.1
XAD/Sox	2.4	6.0	16.0	4.5	5.8	10.3
PUF/SFE	18.4	24.2	15.0	22.0	23.0	12.2
PUF/Sox	6.8	<0.05	11.0	7.7	<0.05	12.4
PUF/SFE	20.7	10.5	18.5	33.6	17.6	18.5
PUF/Sox	2.0	3.3	3.5	trace	1.5	3.0

*Soxhlet Extraction

SOLUTE FOCUSING IN SUPERCRITICAL FLUID CHROMATOGRAPHY

A method of solute focusing in capillary supercritical fluid chromatography, similar to cryogenic focusing in capillary gas chromatography, would be most useful for improved injection methods and for the efficient coupling of supercritical fluid extraction with capillary supercritical fluid chromatography. For some analytes, the relatively high solvating power of the chromatographic mobile phase, even at low densities, is sufficient to create significant chromatographic band broadening during an injection process. This generally precludes injection methods which deposit the analytes over a finite time period and rely on the chromatographic column to focus and concentrate them into a sharp band for subsequent chromatographic separation.

One possible method of achieving solute concentration at the head of an SFC column would be to create a density drop in a short length of the column by heating it to a significantly higher temperature. To evaluate this thermal focusing approach, several preliminary experiments were conducted. The first set of experiments was designed to determine if peak splitting of lower molecular weight alkanes caused by an insufficient split ratio during split injection could be reduced or eliminated by thermal focusing. The initial chromatograms from such an experiment are shown in Figure 22. The top chromatogram was obtained without thermal focusing and the bottom chromatogram was obtained with thermal focusing. The chromatographic conditions for both analyses were similar with operating temperatures of 75°C and initial densities of 0.15 g/mL. Density ramps of 0.025 g/mL were initiated fifteen minutes after the injections. Thermal focusing was accomplished by heating the column inlet to 150°C for one minute prior to injection and then for an additional two minutes following injection. Severe peak splitting was obtained for the entire molecular weight range of the alkane standard compounds in the unfocused analysis. The chromatographic peaks were sharper and no peak splitting was observed for the analysis employing thermal focusing. Although less material was actually injected in the thermally focused analysis (smaller peaks), which could partially account for the improved performance, the thermal focusing approach markedly improved the injection process. The detector and pumping systems were not stable during these analyses which accounts for the baseline shift and slightly different retention times observed between the two chromatograms. Similar results (promising, yet somewhat ambiguous) were obtained with replicate experiments.

The thermal focusing approach was subjected to more intensive evaluation by utilizing it with splitless injection. In this way, more equivalent injections could be obtained and more demanding conditions placed on the focusing requirements. Several operational parameters were also evaluated. They included temperature of the heated zone, length of the heated zone, time duration of the focusing, position of heated zone on the column inlet (e.g., adjacent to the

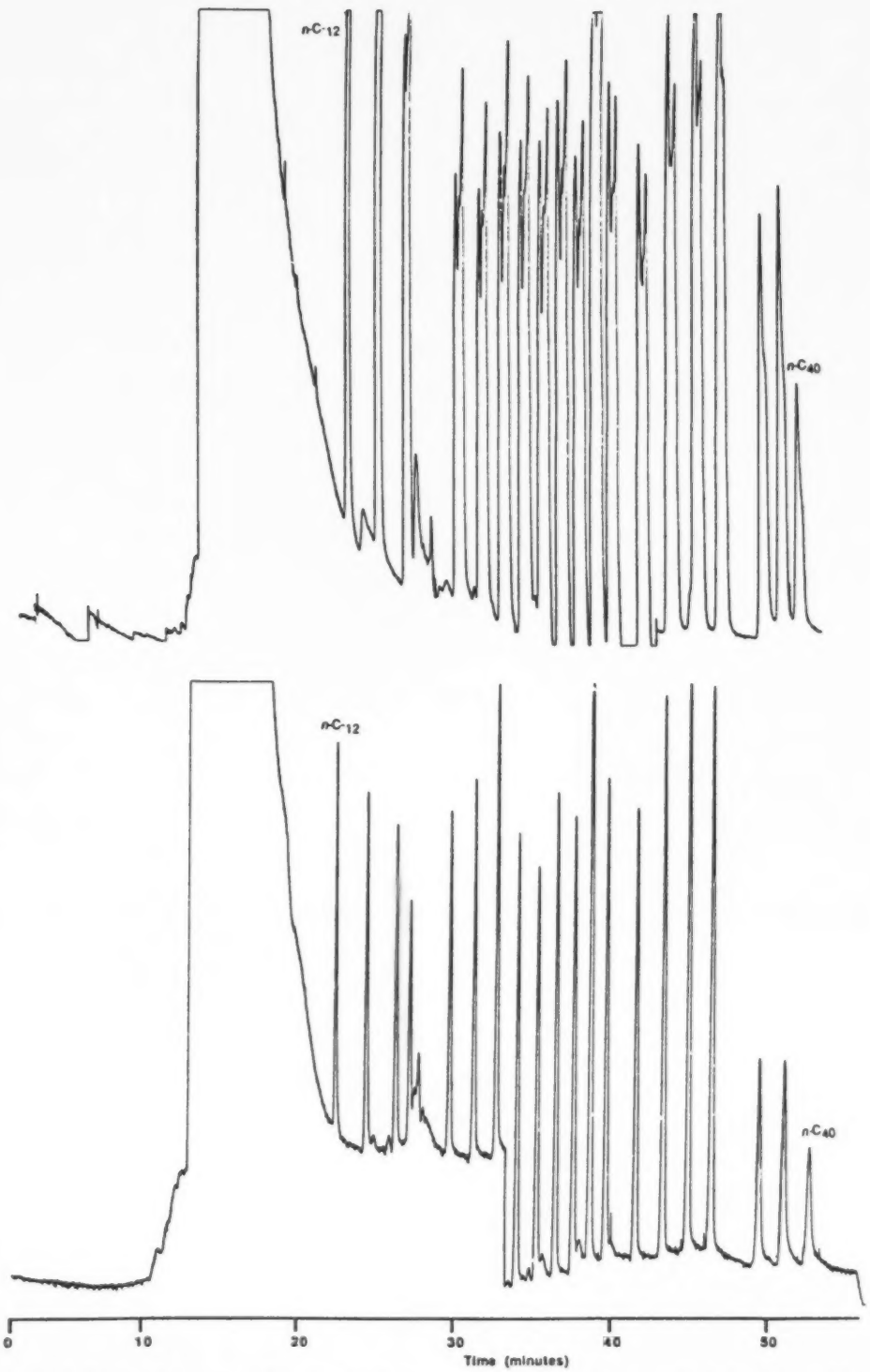

Figure 22. SFC chromatograms of a standard alkane mixture utilizing split injection without (top) and with (bottom) thermal focusing.

injection port or a few cm along the column length), and timing (in relation to the injection) of the heating application. In general, variation of these parameters did not create significant effects. However, slightly better performance was achieved with the following combination of parameters: moderate heating (e.g. 150°C), utilization of the longest heated zone evaluated (8 cm), application of the thermal focusing heat from immediately before the injection until several minutes after the injection, and positioning the heated zone approximately 40 cm along the column length from the injection port. It is reasonable that thermal focusing would be less effective at temperatures higher than 150°C due to the volatility of the alkane analytes. It is also consistent that better focusing was achieved when the heated zone was placed a short distance away from the injection port to allow partial concentration of the solutes in the chromatographic column and passage of the solvent front.

One of the more dramatic examples of the effects of thermal focusing with a splitless injection is shown in Figure 23. A short 5 m x 50 μm i.d. column was used for these analyses with an operating temperature of 75°C and an initial density of 0.15 g/mL (75 bar). A density ramp of 0.025 g/mL was initiated ten minutes after the injection. Thermal focusing was accomplished by heating at 150°C for five minutes beginning immediately before the injection. The 8 cm heated zone was positioned 40 cm from the injection port. The top chromatogram which did not utilize thermal focusing exhibited peak splitting for all but the three highest molecular weight alkane compounds (C_{36}, C_{38}, and C_{40}). No peak splitting was observed for the bottom chromatogram which utilized the thermal focusing method. However, narrower chromatographic peaks were not obtained as would have been expected. Replicate experiments did not show as dramatic effects with the application of thermal focusing, but reduced peak splitting was always obtained. Additional studies are necessary to better understand and to develop improved thermal focusing methods.

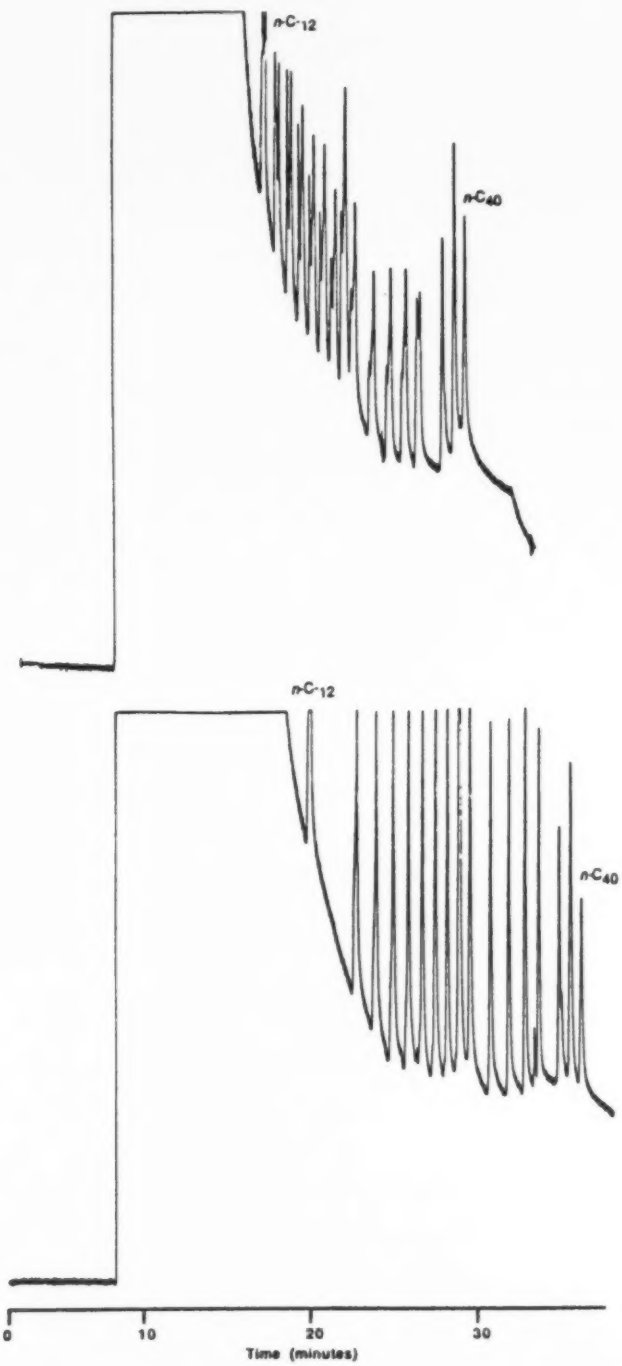

Figure 23. SFC chromatograms of a standard alkane mixture utilizing splitless injection without (top) and with (bottom) thermal focusing.

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